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FERTILISER REQUIREMENTS IN AN ACID PEAT

By T. M. MORRISON* and J. R. CAUGHLEY, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(*Received for publication, 31 August 1959*)

Summary

Spinach plants grown in an acid peat in a glasshouse responded to applications of lime, boron, and copper. The response to copper was not evident until boron was simultaneously added.

INTRODUCTION

There are considerable areas of acid peats in New Zealand in localities in which market gardening could be, or is, the major industry.

One such area, situated on the Lands and Survey Farm, Whateroa block, at Paraparaumu, is classed in set 107, Makerua peaty loam (N.Z. Soil Bureau 1954). Dr W. F. Harris (pers. comm.) has described the type as "a forest peat shallow to moderately deep; non-fibrous woody peat over sedge peat with a margin of peaty silt to peaty sandy loams". In this area the peat is 14–15 ft deep and has been reclaimed from tall manuka. The consolidation is good, the drainage fair, the surface hummocky and moderately stumpy. The analysis of a 3–10 in. sample (Lab. No. S.B. 7298B) is as follows:

pH	4.2	Loss on ignition	83.6%
Total N	1.04%	C/N ratio	47
B.E.C.	120.3 me.%	T.E.B.	31.8 me.%
% B.S.	26	Truog phosphorus	1 mg% P
Exch. Ca	13.2 me.%	Exch. Mg	18.4 me.%
K	0.15 me.%	Na	1.9 me.%
Ash (total)	16.4%	Ash (acid insoluble)	13.0%
Alkalinity of ash	28.2 me.%	Total P	35 mg%
Cations in ash Ca	18.2 me.%	Cations in ash Mg	20.1 me.%
K	0.65 me.%	Na	2.0 me.%

The analysis showed that there is some mineral matter incorporated in the peat and that it is likely to respond to substantial amounts of lime and to potash. The Truog phosphate is low and, although total

* Now Botany Department, University of Otago, Dunedin.

N is high, the C/N ratio indicates that it is not immediately available. Consequently, basic dressings of phosphorus and nitrogen are necessary.

Attempts to grow vegetables on the margins of this area have given disappointing results (W. F. Harris, pers. comm.) and it was thought that a deficiency in minor elements was probably responsible.

Methods

The investigation consisted of two pot experiments carried out in a glasshouse. The peat was broken up by hand and plant debris removed before being potted into glass pots of 3 in. diameter containing 285 g moist peat. Fertilisers were then mixed by hand in a porcelain basin with the top two inches of peat from the individual pots. A preliminary pot experiment had shown that the minimum lime requirement was 18 cwt/acre, while phosphorus equivalent to 8 cwt/acre of superphosphate, nitrogen equivalent to 6 cwt/acre of sodium nitrate, and potassium equivalent to 3 cwt/acre of K_2SO_4 were about optimum amounts of these major nutrients. In the experiments, phosphorus, nitrogen, and potassium were added to all pots as AR grade $NaH_2PO_4.2H_2O$, $NaNO_3$, and K_2SO_4. Calcium when used was added as $Ca(OH)_2$. Boron, manganese, and copper were added as $Na_2B_4O_7.10H_2O$, $MnSO_4.4H_2O$ and $CuSO_4.5H_2O$ respectively. Glass wool was used to assist bottom drainage. Resin-purified water was used throughout for preparation of all solutions and for watering, and all drainage was regularly returned to each pot.

All apparatus which came into contact with soil, or with water used during the experiment, had been washed in 50% HNO_3 and rinsed in resin-purified water.

Spinach, which is highly responsive to manganese, boron, and copper (Lucas 1956), was used as the indicator plant in both experiments. The plants were raised in expanded perlite and transplanted one to each pot one week after germination.

At harvest, shoots were cut off, dried overnight at 100°C, and weighed.

Experiment 1. This experiment consisted of 3 blocks:

(a) No calcium (Ca_0).
(b) Calcium at the rate of 18 cwt lime/acre (Ca_1).
(c) Calcium at the rate of 36 cwt lime/acre (Ca_2).

In addition, each of these blocks was subdivided into 5 treatments:

(1) Receiving boron at 6 lb sodium borate/acre.
(2) Receiving copper at 9 lb copper sulphate/acre.
(3) Receiving manganese at 15 lb manganese sulphate/acre.
(4) Receiving boron plus copper plus manganese at above rates.
(5) No added trace elements

There were four replicates of each treatment. Some pots received ammonium molybdate at the rate of 1 lb/acre, but since no effect could be demonstrated, they are not further considered.

Plants were harvested four weeks after transplanting to the experimental pots.

Experiment 2. This experiment consisted of four blocks:

(a) No calcium (Ca_0).
(b) Calcium at the rate of 18 cwt lime/acre (Ca_1).
(c) Calcium at the rate of 36 cwt lime/acre (Ca_2).
(d) Calcium at the rate of 72 cwt lime/acre (Ca_4).

Each of these blocks was subdivided into five treatments:

(1) No copper.
(2) 9 lb $CuSO_4.5H_2O$/acre.
(3) 36 lb $CuSO_4.5H_2O$/acre.
(4) 15 lb $MnSO_4.4H_2O$/acre.
(5) 9 lb $CuSO_4.5H_2O$ plus 15 lb $MnSO_4.4H_2O$/acre.

Boron was applied to all pots at the rate of either 6 lb or 24 lb sodium borate/acre.

There were three replications of each treatment. Plants were harvested five weeks after transplanting to the experimental pots.

RESULTS

Experiment 1.

Dry weights of spinach shoots are shown in Fig. 1. All plants were moribund in the absence of lime and, when lime was added, in the absence of boron. Boron when added with lime increased the dry weight at both levels of applied calcium. The combination of boron, copper, and manganese produced a greater increase in dry weight than did boron alone. The response to this combination of elements was somewhat greater at the higher than the lower level of calcium.

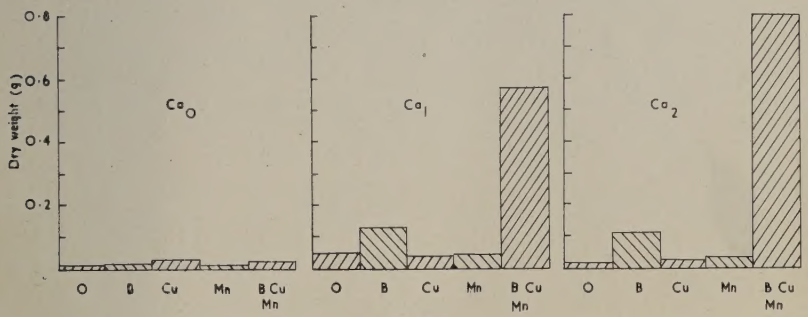

Fig. 1.—Dry weight of spinach shoots after 4 weeks growth in peat. All plants received basal dressing of N, P, and K. Mean of 4 plants.

The second experiment was designed to determine if both copper and manganese were active in promoting growth separately in the presence of boron and to explore further the optimum level for response for the individual minor elements.

Experiment 2.

Plants grown without lime were moribund, as in the previous experiment. Manganese had no effect on growth in any lime treatment

TABLE 1. DRY WEIGHT (g) OF SPINACH SHOOTS AFTER 5 WEEKS GROWTH IN PEAT
All plants recived a basal dressing of N, P, K. (Mean of three plants)

	Lime added			
	Ca_0	Ca_1	Ca_2	Ca_4
B_1 Cu_1	0.005	0.932	0.908	1.017
B_1 Cu_1 Mn	0.005	0.600	0.772	0.935
B_1 Mn	0.004	0.161	0.235	0.180
B_1 Cu_4	0.004	0.799	0.991	1.070
B_1 Cu_4 Mn	0.005	0.871	0.987	1.066

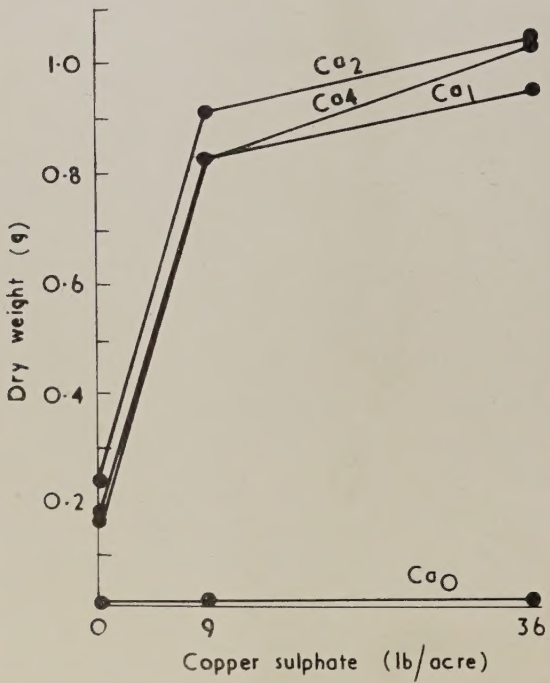

Fig. 2.—Dry weight of spinach shoots after 5 weeks growth in peat. All plants received basal dressing of N, P, K, and B. Mean of 3 plants.

(Table 1). The presence of added manganese has therefore been neglected in discussing the remainder of the experiment.

In this second experiment there was no growth simulation due to raising the levels of lime above 18 cwt/acre (Fig. 2). However,

the response in growth to copper added at the rate of 9 lb $CuSO_4.5H_2O$/acre in the presence of added boron was again evident, but adding greater quantities of copper did not appreciably stimulate any further growth. Similarly, increasing the level of added boron above 6 lb sodium borate/acre had little further effect on growth, although small increases were recorded in the presence of the lowest level of copper and no change or a decrease in dry weight in the presence of the highest level of copper (Table 2).

TABLE 2. DRY WEIGHT (g) OF SPINACH SHOOTS AFTER 5 WEEKS GROWTH IN PEAT
All plants received a basal dressing of N, P, K. (Mean of three plants)

	Lime added			
	Ca_0	Ca_1	Ca_2	Ca_4
B_1 Cu_1	0.005	0.827	0.908	0.826
B_4 Cu_1	0.004	0.932	1.038	1.017
B_1 Cu_4	0.004	0.944	1.040	1.030
B_4 Cu_4	0.004	0.799	0.991	1.070

DISCUSSION

Responses to copper applications by plants in New Zealand have often been recorded and are to be expected in acid peats such as that used here. Thus, Adams (1949) has recorded significant responses to copper sprays by onions grown in an acid peat near Christchurch, and Elliott and van der Elst (1956) found large responses to copper in a variety of plants grown in acid Rukuhia peat. Boron responses are, however, usually associated with a native deficiency in the parent material or with over-liming, although the availability of this element does decrease greatly below pH 5.0 (Truog 1946).

Addition of lime is necessary for plant growth in this peat but normal crops will be produced only with the simultaneous application of both copper and boron.

We wish to thank Dr W. F. Harris for bringing the problem to our notice and for collecting the peat.

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ORGANIC MATTER STABILITY AND NUTRIENT AVAILABILITY IN TAUPO PUMICE

By R. H. Jackman, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 14 October 1959*)

Summary

Soils derived from rhyolitic Taupo pumice when under permanent pasture accumulate organically bound nutrients in the top soil to a high degree. The available nutrient supply is thereby reduced.

Respirometry studies show that this accumulation is not due to a reduced microbial activity resulting from a deficiency of a major or minor nutrient element.

Raising the pH increases the microbial activity of the soil and the rate of breakdown of the organic matter. These increases are due to a release of carbon into an available form. Available carbon is the limiting factor to microbial activity though the soil may contain 12% total carbon.

The inference is made that the stability of the organic matter in Taupo pumice soils is due to the formation of a stable complex. The possible nature of this complex is discussed.

A greenhouse experiment showed that raising the pH releases useful amounts of nitrogen into available form but that a minor-element deficiency can be easily induced. The effect of raising the pH on the mineralisation of organic phosphorus is uncertain.

The agronomic significance of these findings is discussed.

Introduction

In studies on the nutrient economy of grasslands, Walker (1955, 1956) has pointed out that under New Zealand conditions large accumulations of organic matter and organically bound nutrients are to be expected. It is generally recognised that mineralisation of organically bound nitrogen, phosphorus, and sulphur must take place to render them available to plants. Consequently an increase in organic forms must be at the expense of more available forms. It is therefore possible that in soils of low nutrient supply, but in which organic matter accumulates rapidly, an increase in organic matter may result in reduced nutrient supply and pasture production. Even if this extreme situation is not the case, there must still be a reduction in effectiveness of applied phosphate and the nitrogen fixed by clovers.

Soils derived from Taupo pumice appear to exemplify this. Taupo rhyolitic pumice was deposited about 1,750 years ago and the various ash showers have been described by Baumgart (1954). The soils that have developed have affinities with the Ando soils of Japan (Aomine and Yoshinaga 1955) and have been described elsewhere (Taylor and Pohlen 1954). Field evidence (Smallfield 1933; During 1956) has shown that large amounts of topdressed phosphate are necessary to establish and maintain good pastures. After sowing down a new pasture following ploughing, there is a large accumulation of organic matter in

the top soil (Browning and Baumgart 1954; Thapa 1956) while considerable biological fixation of applied phosphate has been demonstrated (Jackman 1951).

The high organic-matter content does not appear to be related to factors commonly associated with this condition, for the rate of input of plant material is not unduly high nor can these pasture residues be considered particularly resistant to decomposition. Excessive acidity, poor drainage or low temperatures are not implicated as major factors. The mineral colloids of these soils are amorphous to X-rays and have been described by Fieldes (1955) as allophane B. He obtained evidence that organic matter was complexed with these colloids and was resistant to oxidation by H_2O_2. Jackman (1955) has suggested that such complexes are resistant to microbial attack, an opinion shared by several Japanese workers (Hosoda and Takata 1953; Kosaka and Honda 1955) working with allied soils derived from andesitic ash. Aomine and Kodama (1956) have shown that such amorphous colloids may retard the decomposition of albumin and cellulose to a greater extent than a mixture of illitic/montmorin clays.

The present work is concerned with obtaining evidence as to whether such resistant complexes occur in Taupo pumice or, alternatively, whether the high levels of organic matter can be due to a reduced microbial activity resulting from a nutrient deficiency.

MATERIALS AND METHODS

Taupo sandy silt was selected as being a soil representative of the group. Samples of the 0–2 in. layer under 2- and 24-year pastures were collected. The 2-year pasture had been sown on ploughed virgin ground. The soil under the older pasture was selected because it had the highest organic-matter content, for this soil type, known to the author. The two samples are representative of a very young and a mature stage in the progressive accumulation of organic matter in the top soil. The samples were sieved (5 mm), partially air dried, bottled,

TABLE 1. ANALYSIS OF TAUPO SANDY SILT UNDER PASTURES OF DIFFERING AGE

Pasture Age, years		2	24
C.E.C.	me./100 g	14.6	33.6
Exchangeable Ca,	me./100 g	3.4	14.2
Mg,	me./100 g	0.24	2.6
K,	me./100 g	0.19	1.6
Na,	me./100 g	0.10	0.6
% saturation		27	57
C%		4.6	12.0
N%		0.31	1.05
C/N		14.8	11.4
Total P%		0.114	0.175
Organic P%		0.059	0.095
Volume weight, lb/1 in. acre		150,000	

and placed in the refrigerator (2–4°C) until required. Analyses of the two samples are given in Table 1.

Relative biological activity was measured by incubating 50 g of soil adjusted to 70% of the moisture-holding capacity in a modification of a Swaby and Passey macrorespirometer (1953). Initially both oxygen consumed and carbon dioxide evolved were measured but it soon became evident that either measurement gave similar relative activities, and carbon dioxide measurements were discontinued. The incubation temperature was 28 ± 2°C. In later experiments a Warburg respirometer was used.

RESULTS

Experiment 1. Oxygen Uptake and Soil pH

Soil pH was adjusted with dilute NaOH or solid $Ca(OH)_2$. A parallel series was set up in mason jars and was periodically sampled for pH measurements (soil : water = 1 : 2.5). Oxygen consumed as a function of soil pH is plotted in Fig. 1. There was a marked fall in pH during incubation especially in the case of the 24-year pasture soil but no attempt has been made to average pH values over time. The pH values used were measured at the end of the incubation period.

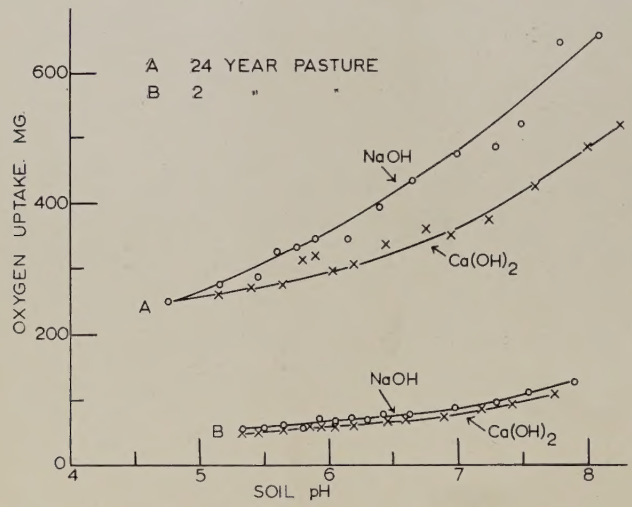

Fig. 1.—Oxygen uptake of Taupo sandy silt from under 2- and 24-year-old pastures with pH adjusted by additions of NaOH or $Ca(OH)_2$.

Increases in pH were accompanied by increases in oxygen uptake. Sodium hydroxide was more effective than $Ca(OH)_2$. In a similar study using the 24-year pasture soil, the pH was adjusted with NaOH but to one series 1.5 me. of $CaCl_2$ were added to each 50 g of soil. At comparable pH values the $CaCl_2$ consistently reduced the oxygen uptake.

Since no measurements were made within 48 hours of setting up the experiment it is not surprising that cumulative oxygen uptake curves showed no evidence of a period of adjustment by the population. While adjustment may have taken place it was apparently accomplished quickly since large increases in oxygen uptake were seen within 2 hours of adding the amendments.

Experiment 2. Nitrogen, Phosphorus, Lime Factorial. Two-year Pasture Soil

In this and the subsequent experiment additions are given as mg per 50 g of soil. 100 mg is equivalent to 300 lb per acre inch.

Treatments

1. NH_4NO_3 at 100 mg.
2. H_3PO_4 titrated with NaOH to pH 5.5 (that of the soil); 11.64 mg P.
3. $Ca(OH)_2$ at 300 mg.

Four replicates were incubated for 384 hours when pH measurements were made on two replicates. The results are given in Table 2. Since the treatments produced differing soil pH values the nutrient effects are confounded. A correction for pH differences has been made in the following manner. Experiment 1 had been continued for 430 hours when the control had absorbed 97 mg of oxygen comparable to the 99.8 absorbed in the present experiment. Soil pH changes had been found to be negligible after 300 hours incubation. An equation was fitted to the data of Exp. 1. The relationship was linear. From this equation, estimates of oxygen uptake at desired pH values and associated errors were obtained. Standard errors were then found for the present experiment and also for the differences between oxygen uptake found and that predicted from the soil pH values. These differences are considered to be effects due to nutrients.

TABLE 2. OXYGEN UPTAKE AND FINAL pH OF THE TWO-YEAR PASTURE SOIL TREATED WITH P, N, AND $Ca(OH)_2$.

Treatment	Soil pH	Oxygen Uptake, mg/50 Soil	
		Total	Due to Nutrient*
Nil	5.17	99.8	+ 5.6
P	6.10	137.0	+13.5
N	5.19	92.5	− 2.5
Ca	7.25	155.8	− 6.9
PN	5.67	94.5	−15.4
PCa	7.50	194.0	+22.7
NCa	6.50	164.0	+27.3
PNCa	6.45	184.0	+49.0
S.E.	—	± 2.8	± 4.8

* After removal of pH effect.

No nutrient used alone, with the possible exception of phosphorus, increased the oxygen uptake significantly. Calcium and nitrogen may have decreased the demand for oxygen. However, significant increases

in oxygen uptake were obtained when calcium was used with nitrogen or phosphorus. The greatest uptake occurred when all three elements were present.

Experiment 3. Major and Minor Elements. Twenty-four-year Pasture Soil

Treatments

1. Nil.
2. KH_2PO_4, 17.44 mg P (22 mg K)
3. KH_2PO_4, plus Minor Elements (M.E.)*
4. KH_2PO_4, plus M.E. plus $NaNO_3$, 37.3 mg
5. KH_2PO_4, plus M.E. plus $Ca(OH)_2$, 146 mg
6. KH_2PO_4, plus M.E. plus $Ca(OH)_2$, 278 mg
7. KH_2PO_4, plus M.E. plus $NaNO_3$, 37.3 mg plus $Ca(OH)_2$, 146 mg
8. KH_2PO_4, plus M.E. plus $NaNO_3$, 37.3 mg plus $Ca(OH)_2$, 278 mg

Four replicates were incubated 500 hours when pH measurements were made. The results are given in Table 3. Correction for variable pH values have been made as previously. Again the relationship between pH and oxygen uptake was linear over the pH range of the present experiment.

TABLE 3. Oxygen Uptake and Final pH of the 24-year Pasture Soil Treated with Major and Minor Elements

Treatment	Soil pH	Oxygen Uptake, mg/50 g soil	
		Total	Due to Nutrient*
1. Nil	5.16	561	138
2. KP	5.25	622	193
3. KP, M.E.	5.16	553	130
4. KP, M.E., N	5.16	512	89
5. KP, M.E., $Ca(OH)_2$	5.87	607	128
6. KP, M.E., $Ca(OH)_2$	6.33	658	132
7. KP, M.E., N, $Ca(OH)_2$	5.86	599	121
8. KP, M.E., N, $Ca(OH)_2$	6.40	617	83
S.E.		± 14	± 21

* After removal of pH effect.

While the nutrient effects suggest an increased oxygen uptake due to KH_2PO_4 and a reduced uptake due to nitrogen, no treatment achieved an effect significantly different from control.

In a subsequent experiment the minor elements were again tested as the full mixture and also subdivided into six differing groups. No mixture produced any change in oxygen uptake when compared with control.

The pH effect was then studied. The observed early effect from raising the pH suggested that the mechanism was chemical rather than biological and one concerned with the organic matter substrate rather than the microbial population itself.

* A "minor element" mixture of Zn, Cu, Fe, Mn, Co, Ni, at 10 lb per acre (150,000 lb) of the sulphate or chloride, borax at 10 lb, WO_3, VO_3 at 1 lb, sodium molybdate at 4 oz, and epsom salts at 100 lb.

Experiment 4. Auto-oxidation

Bremner (1949) has shown that auto-oxidation of soil organic matter takes place at elevated pH values. This possibility was studied.

Air-dried soil was partially moistened and sterilised with $HgCl_2$ or propylene oxide—5 ml of 1% $HgCl_2$ or 1 ml of propylene oxide per 20 g of dry soil. Two levels of NaOH were used. The treatments were applied in the morning and the oxygen uptake of the $HgCl_2$ series measured in the afternoon using the equivalent of 1 g of dry soil in a Warburg respirometer. The propylene oxide series were similarly studied next day. The results are given in Table 4. It is evident that over a pH range where large increases in oxygen uptake are found, auto-oxidation is of small importance.

TABLE 4. OXYGEN UPTAKE OF THE 24-YEAR PASTURE SOIL TREATED WITH SODIUM HYDROXIDE WITH AND WITHOUT MERCURIC CHLORIDE OR PROPYLENE OXIDE

Treatments	Soil pH and Oxygen Uptake, μl/g soil/min			
	Mercuric Chloride		Propylene Oxide	
	pH	Oxygen	pH	Oxygen
Nil	5.1	0.678	5.1	0.263
Hg or P.O.	4.7	0.000	5.4	0.000
NaOH	6.2	1.159	6.3	0.491
NaOH plus Hg or P.O.	5.7	lost	6.6	0.045
NaOH	7.5	1.600	7.5	1.100
NaOH plus Hg or P.O.	7.1	0.060	7.7	0.098

Experiment 5. Sucrose/pH Interaction. Twenty-four-year Pasture Soil

A preliminary experiment had shown that if the 24-year pasture soil was removed from the refrigerator after several months storage, stood at room temperature and then incubated with sucrose added, there was an immediate and large increase in oxygen uptake due to the sucrose. On the other hand, if the soil was air-dried, stored dry, and then moistened and incubated, the oxygen uptake was very much higher and was not increased by the addition of sucrose.

These observations suggested that the limitation to microbial activity was an inadequte supply of available carbon despite the fact that the soil contained 12% total carbon. If raising the pH was in fact releasing carbon into an available form, then it follows that, at the original pH, added sucrose should increase the oxygen uptake, but that this effect should be decreased or eliminated if the pH were raised. Two necessary experimental conditions would be that excess available carbon should have been removed by a prior incubation, and that measurements be made before there had been time for the microbial population to change due to treatment. This hypothesis was tested.

Soil that had been stored moist at room temperature for four weeks was treated with dilute NaOH with and without 2% sucrose. Nitrogen was added to one sucrose treatment. Moisture was adjusted to 70% of the moisture-holding capacity and the equivalent of 1 g of dry soil was placed in Warburg flasks. The first reading was made $1\frac{1}{4}$ hours after applying the treatments. The results are plotted in Fig. 2.

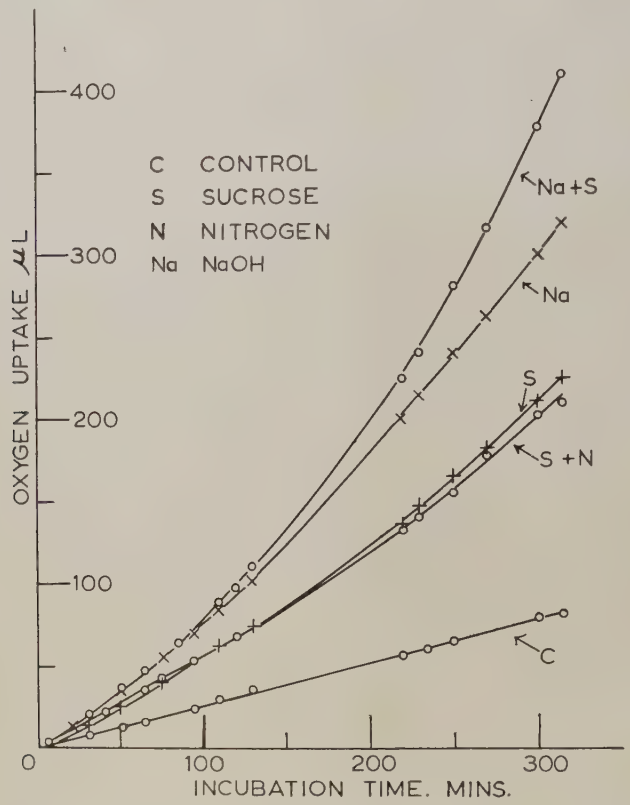

Fig. 2.—Oxygen uptake of Taupo sandy silt from under a 24-year-old pasture when treated with sucrose, nitrogen, and NaOH.

The uptakes were curvilinear excepting that for the control, but for the first 80 minutes were essentially linear, indicating that over this period the microbial population was relatively stable. The rates of oxygen uptake over this early period were calculated and are given in Table 5. It is seen that sucrose increased the oxygen uptake in the absence of sodium hydroxide but had essentially no effect for the first 80 minutes when the pH was raised. It can be inferred that raising the pH did release carbon into an available form and thereby remove the primary limitation to microbial activity. With time, the microbial activity entered a logarithmic phase and was greatest when sucrose

TABLE 5. RATES OF OXYGEN UPTAKE DURING THE FIRST 80 MINUTES INCUBATION OF THE 24-YEAR PASTURE SOIL TREATED WITH SODIUM HYDROXIDE WITH AND WITHOUT 2% SUCROSE

Treatment	Soil pH	Oxygen Uptake, μl/g soil/min	
		Found	Due to Sucrose
1. Nil	4.8	0.24	—
2. Sucrose	4.8	0.57	0.32
3. Sucrose plus N	4.8	0.55	
4. NaOH	7.5	0.76	—
5. NaOH plus sucrose	7.3	0.77	0.01

was present at the higher pH. This development indicates that with the raised pH, carbon was still the limiting factor to microbial activity.

Experiment 6. Soil pH and Available N. Twenty-four-year Pasture Soil

Since raising the pH of the soil increased the mineralisation of carbon, nitrogen also should be mineralised and in considerable amounts from a soil containing 1% (Thomson *et al.* 1954). Experience however has failed to show that liming pumice soils in any way improves pasture growth. An induced minor-element deficiency appears a likely reason but it is also conceivable that mineralisation of nitrogen may be ephemeral or slight. A pot experiment was set up to obtain information on these points.

Bitumen-painted earthenware pots holding 2.1 kg of air-dry soil were used. Glass-distilled water was used to keep the soil at 60% of moisture-holding capacity. There were 5 replicates in a randomised block design. Two series were set up, one planted to subterranean clover and the other to short-rotation ryegrass. The treatments were as follows:

(a) *Subterranean clover*

1. Nil
2. $Ca(OH)_2$ at 9.45 g per pot initially and a further 7.5 g at replanting, both being mixed throughout the soil.
3. Minor elements (M.E.). Cu, Zn, Mn as sulphates at 10 lb per acre and borax at 20 lb, watered on initially and at replanting.
4. $Ca(OH)_2$ and minor elements similarly applied.

(b) *Short-rotation ryegrass*

Basal 1. Urea at 50 lb per acre initially, after the first cut and at replanting.
2. KH_2PO_4 at 200 lb per acre at replanting. Both were mixed into the top inch of soil.

Individual $Ca(OH)_2$ and minor elements as for the clovers.

The initial lime raised the soil pH to 6.2–6.3. Since a greater minor-element stress was desired, the plants were removed, the additional lime was added and the soil was then repotted and replanted.

The results of the clover experiment are given in Table 6. Liming increased the growth of the first planting and the increase was highly significant. There was also a highly significant interaction between

lime and minor elements, the minor elements increasing growth only in the presence of lime. With the second planting lime caused a highly significant decrease in growth and again there was a highly significant interaction between lime and minor elements. These results are consistent with those of McNeur (1949) and indicate that liming can easily induce a minor-element deficiency in this soil. The better growth of the clovers of the first planting which received lime was probably due to two causes. First, the minor-element deficiency was not so acute as in the second planting and, second, there was a useful release of available nitrogen inferred from the observation that those plants growing in the limed pots were poorly nodulated as compared with those growing in the unlimed pots.

TABLE 6. Dry-matter Yields of Subterranean Clover Grown in Treated Taupo Sandy Silt

	Treatments				Sig. diff. at 0.01 level
	Nil	$Ca(OH)_2$	M.E.	$Ca(OH)_2$ + M.E.	
			g/pot		
First planting	5.30	6.42	4.81	7.63	0.66
Second planting	8.06	3.64	7.12	6.18	1.94
			Soil pH at harvest		
First planting	5.0	6.2	4.9	6.1	—
Second planting	5.5	7.2	5.5	7.2	—

In Table 7 are given the results of the ryegrass experiment. After the first cut of the first planting it was observed that all plants in the pots without lime were light in colour. The additional nitrogen restored the colour but was probably given too late to affect the growth. While it is significant that one cut of ryegrass largely exhausted the supply of available nitrogen, the results are not given because of uncertainty of the effect of the second application of nitrogen.

Lime decreased the growth of the first cut significantly, but minor elements increased growth significantly. In the second and third cuts, lime increased growth significantly but minor elements had no effect. The nitrogen content of the grass at each cut was higher in those plants grown with lime than in those grown without lime. These results show that under glass-house conditions the first factor limiting the growth of ryegrass was nitrogen and that if this were supplied (cut 1) then at a pH of 7.2 a minor-element deficiency became limiting. They also show that liming did increase the uptake of soil nitrogen, presumably a reflection of increased mineralisation. The increase was 163 mg or 67% more than the amount of nitrogen removed from the unlimed soil.

TABLE 7. DRY-MATTER YIELDS AND NITROGEN CONTENTS OF AND UPTAKES BY SHORT-ROTATION RYEGRASS GROWN IN TREATED TAUPO SANDY SILT

Treatments	Nil	M.E.		$Ca(OH)_2$	$Ca(OH)_2$ + M.E.		$Ca(OH)_2$			
	Dry matter, g/pot						Nil		Present	
							N%	N mg	N%	N mg
Second Planting			Total			Total				
Cut 1	5.83	6.55	12.38	4.03	4.95	8.98	3.34	207	3.81	166
Cut 2	3.08	2.99	6.07	4.97	5.79	10.76	2.05	62	3.58	193
Cut 3	2.82	3.03	5.85	5.49	4.95	10.44	1.99	58	2.56	134
Sig. diff. 0.01 level: cut 1, 0.70; cut 2, 0.93; cut 3, 0.69.										
Nitrogen uptake, cuts 1–3, mg/pot								327		493
Nitrogen added at planting, mg/pot									84	
Soil nitrogen, uptake, mg/pot								243		409

Discussion

Nutrient Deficiency

The interpretation of the results of Exp. 2 on possible nutrient deficiencies must be made in conjunction with the results of Exp. 5 where it was shown that raising the pH increased the supply of available carbon. In Exp. 2 the direct pH effect was removed but there remains the fact that associated with the rise in pH there was an increase in available carbon. Table 2 shows that positive effects were small or non-existent when phosphorus, nitrogen, or calcium was used singly but that when calcium hydroxide was used in conjunction with phosphorus or nitrogen, and especially when both were present, positive increases were found.

The probable explanation is that the first factor to limit microbial activity in the two-year pasture soil was available carbon. At this low level of available carbon, nitrogen and phosphorus were adequate. The use of calcium hydroxide increased the supply of available carbon to a higher level at which both nitrogen and phosphorus supplies were inadequate. Consequently activity was increased to the greatest extent when all three treatments were used. The results indicate that nitrogen became limiting before phosphorus. The possible effect due to phosphorus alone is similarly explained because this treatment was accompanied by a rise in pH. The increased available carbon, together with the added phosphorus, combined to increased biological activity.

Experiments 3 and 5 show that in the 24-year pasture soil, as in the 2-year pasture soil, a lack of available carbon limited microbial activity.

One can only conjecture why calcium and nitrogen when used alone tended to reduce the oxygen uptake (Fig. 1 and Tables 2 and 3). Demolon and Barbier (1929) have shown that calcium increases the mutual flocculation of humic acid and the clay fraction from "fire clay", and one would infer from the work of Bremner and Lees (1949) on the pyrophosphate extraction of organic matter that divalent cations should stabilise organic matter. It was noticed that if samples treated with sodium or calcium hydroxide had water added to them and this water was then squeezed out, the water from the sodium-treated soil was darkly coloured, but that at a comparable pH the water from the calcium-treated soil was clear. Flocculation due to calcium may have reduced the active surface area of the organic matter and the opportunity for biological attack.

The depression due to nitrogen may be an artefact of respirometry when continued over relatively long periods of time. During one experiment, half the respirometer chambers were replaced by others having a larger volume. Identical treatments happened to be used with each set of respirometers and it was found that the oxygen uptake in the larger units was consistently greater than in the smaller. There appeared to be no reason for differences in ease of gaseous exchange between the two types of chambers. It is possible that, during incubation, toxic or inert gases are produced which gradually accumulate. The concentration of such a gas would be greater in the smaller

chambers and the depression of the oxygen measured consequently greater. It has been shown (Wijler and Delwiche 1954; Jannson and Clarke 1952) that some degree of denitrification is possible under aerobic conditions and is favoured by the presence of nitrate and organic matter. These conditions were present in the samples to which ammonium nitrate had been added and it is possible that the production of gaseous nitrogen was responsible for the apparent lowered oxygen demand where nitrogen was used.

The results show that microbial activity was in the first instance limited by a carbon deficiency in both soils but that if carbon were made available as a result of raising the pH, then there was a deficiency of nitrogen and phosphorus in the two-year pasture soil but no nutrient deficiency in the soil carrying the older pasture. It is inferred that the stability of the organic matter in these soils is not due to a nutrient deficiency which limits microbial activity; further that the pH effect is not due primarily to a resultant increase in the availability of the nutrients tested.

The pH Effect

A change in pH can increase microbial activity through a number of differing mechanisms the reduction of a toxicity, a change in the environment suitable for a larger or more active population, the approach to an optimum pH for the enzymes involved, or it can act on the substrate and change it into a form chemically or physically more available. No known reason exists for the immediate increase in activity of a microbial population when sucrose is added other than that available carbon has been supplied. This is the situation found in Exp. 5. Sucrose can apparently rejuvenate an existing population in much the same way as the wetting of a dried soil as found by Birch (1958). The obvious conclusion is that the stability of the organic matter in Taupo pumice is due to the immobilisation of the organic matter in such a way that lack of available energy-material limits microbial activity.

The stability of soil organic matter has been studied extensively. Waksman and Iyer (1932) proposed a resistant ligno-protein complex. Tyulin (1938) fractionated the organic matter into two fractions of which one is more resistant, is a criterion of infertility rather than fertility, and is found more especially in weathered soils having free and active sesquioxides. Numerous workers have studied the adsorption of proteins by layer lattice clay minerals. They have found that in general proteins are more firmly held than carbohydrates and that the expanding clays hold more than the kaolinitic types since, in the former, inter-layer adsorption takes place. Adsorbed organic groups, especially when in an inter-layer position, are protected from enzymatic attack (Lynch and Cotnoir 1956; Mortland and Gieseking 1952), while enzymes being proteins are adsorbed and their activity reduced (Mortland and Gieseking 1952). Mattson (1932) has propounded systems of isoelectric precipitation to account for the stability of organic matter. A different viewpoint is given by Handley (1954) who found in mor soils cellulose fibres coated with stabilised proteins. Stabilisation was

due to such tanning agents as polyphenols whose presence was dependent upon the type of vegetation.

Studies on the effect of pH have shown that with rising pH adsorption by lattice clays is generally reduced in the case of proteins but the pH effect is small in the case of carbohydrates. It appears that those materials possessing potentially cationic nitrogen groups are adsorbed more readily and their degree of adsorption is greatly dependent upon pH. A rise of pH decreases the positive charge or, if the isoelectric point is exceeded, increases the negative charge, and since lattice clays are electro-negative, interaction is reduced.

The results obtained indicate that in Taupo pumice there is a stable form of organic matter which becomes less stable as the pH of the soil is raised. Instability is not restricted to any particular range of pH though it tends to increase rather more rapidly once a pH of about 6.5 is reached (Fig. 1).

Although these results do not in themselves show whether the stable form is an organic/organic or an organic/mineral colloid complex, there is presumptive and one experimental piece of evidence suggesting that the latter is important. Aomine and Kodama (1956) isolated the clay from an ando and two alluvial soils. The ando colloid was X-amorphous and was considered to be primarily allophane while the alluvial clays were illites with some vermiculite and montmorillonite. These clays were incubated with cellulose or albumin with basal nutrients and three levels of phosphate. The residual albumin carbon after 30 days incubation are given below:

C remaining as % of C added

KH_2PO_4 mg/g colloid	Soil	
	Alluvial (av)	Ando
0	27	74
4	23.5	63
10	24	59

While the amorphous colloid provided much the greater protection, phosphate reduced this effect partially and the authors point out that phosphate deficiency may have limited microbial activity. On the other hand, as has been found in the present work, such systems are very sensitive to changes of pH and the effect of the phosphate may have been due to the KH_2PO_4 causing a slight rise in pH.

There is considerable presumptive evidence linking the sesquioxides with stable organic matter. Aluminium has been found associated with organic matter extracted with metal complexing reagents (Martin and Reeve 1955) and in the supernatant liquor of volcanic ash soils extensively treated with H_2O_2 (Fieldes 1955). Hosoda and Takata (1953) found that Tyulin's "group 2" colloids (isoelectric and associated with

sesquioxides) were very high in allophanic soils. Kawaguchi *et al.* (1954) noted that volcanic ash soils high in organic matter show a marked rise in pH when treated with neutral sodium fluoride—the hydrated alumina is complexed and hydroxyl is liberated. Aluminium humates but not iron humates show this reaction. This was confirmed by the author in the case of Taupo pumice (pH 10.5) and samples of yellow-brown loams from andesitic ash (pH 11.1 11.5) both of which contain amorphous allophanic colloids; but soils having developed lattice clay minerals did not exceed pH 9.5.

The work on lattice clay minerals, especially that of Pinck *et al.* (1954) has shown that adsorbed organic materials are only strongly protected against enzymes if they are monolayers in an inter-layer position. Protection appears to be due to physical impedance. With amorphous colloids this type of protection does not appear to be possible unless the organic particles are coated with the mineral colloid. The colloid particles are very small—the surface area has been given as 400 m^2/g (Gradwell and Birrel 1954)—which might allow of this happening.

Such physical impedance is not the only way in which organic materials can be protected against microbial attack. In the tanning of leather, the tanning agents (chromium or polyphenolic) form cross links between carboxyl and amino groups of protein chains and the greater the degree of cross linking the greater the stability against heat and enzymes (Gustavson 1949). While in practice basic chromium sulphates are used, other polyvalent metals, including aluminium, possess this linking ability to an appreciable extent. Gustavson also points out that by chroming collagen and thereby activating basic groups, the speed of reaction between the polyphenolic tannins and the collagen is greatly increased.

Handley's findings suggest that in mor soils there is a parallelism between protein tanning and the stabilisation of organic matter under such conditions. Leaf proteins could be precipitated by water extracts from leaves which showed some of the chemical reactions of tannins and such precipitates were in varying degree resistant to decomposition by fungi. He obtained some evidence that precipitation is facilitated by more-acid reactions and that this acidity effect was not due to isoelectric effects. He further points out that tanned leather waste has little fertilising value but that by treatment with alkali it changes to a less stable form and has manurial value. It is possible that in Taupo ash soils a process akin to tanning activated by the presence of aluminium stabilises part of the organic matter. There is no direct evidence for this hypothesis. If it occurs, a corollary is that that portion of the soil organic matter so stabilised should be high in nitrogen. This conclusion is in general agreement with the present findings. When the 24-year pasture soil had sucrose added (2%) and the pH was raised (Exp. 5), carbon was still the limiting factor. Further, from Exp. 6 it is evident that, although there is plenty of nitrogen in the soil, only a very small portion is available to plants. This hypothesis would explain an apparent anomaly between the present findings and those of Bailey (1953) who found that the cellulose

and hemicellulose contents of Taupo soils were higher than those of some other mineral soils. These carbohydrates may be stabilised by tanned protein as suggested by Handley.

However, Aomine and Kodama's results show that some other mechanism is probably the more important. Isoelectric precipitation can at least initiate interactions between alumina and organic matter. This interaction will tend to decrease with increase in pH. Fieldes (1958) found that between pH 6–7 the increase of the negative charge on Taupo colloid is very large—the variable charge is much greater than is found in lattice clays. Above this pH range there should be a decrease in adsorption of organic matter. Fig. 1 shows that up to about pH 6.5 the oxygen demand when plotted against pH was nearly linear, but above this pH the rate of demand of oxygen progressively increased. This effect was very marked during the early stages of incubation but decreased with time. While the shape of the curve is probably the resultant of a number of variables it does not contradict the concept of this type of precipitation.

Agronomic Significance

Table 1 shows that over 22 years under pasture the increases in total and organic phosphorus were 0.061% and 0.036% respectively. Nitrogen increased by 0.74%. These figures correspond to increases of 183 and 108 lb per 2-inch acre of total and organic phosphorus (P) respectively and 2,220 lb of nitrogen. If the increase in total phosphorus is considered to be due to fertiliser phosphorus, then about 60% of that phosphorus had been converted into organic form and thereby immobilised. Averaged over the period, the annual immobilisation of phosphorus and nitrogen was 4.9 and 101 lb per 2-inch acre respectively. A further, though smaller immobilisation, would be expected at depths greater than 2 inches. This comparison is between only two samples but the results are in general agreement with the author's unpublished findings which have been based on numerous comparisons.

It is characteristic of these soils that the pastures are strongly clover-dominant during the early years following sowing down. This is due to there being insufficient nitrogen to give vigorous grass growth. Again if in later years phosphate topdressing is not maintained, inevitably the clovers fail and, as the nitrogen supply falls, ryegrass is replaced by those species which can exist on a low supply of nitrogen.

In the light of these experiences the accumulations of nitrogen and phosphorus in organic form may be important losses in actual available nutrients; they may of course be gains in potential fertility which may be used in later years to grow a row crop. Consequently any decrease in the rate or extent of accumulation of these organically bound nutrients could mean a corresponding increase in the actual productivity of these soils when growing a pasture.

The results suggest that liming the pumice soils should reduce the immobilisation of fertiliser phosphorus and nitrogen fixed by clovers and release useful amounts of these nutrients into available forms. Exp. 6 shows that useful amounts of nitrogen are in fact released by raising the pH. From the results of Thompson *et al.* (1954), one would

expect that raising the pH would have a relatively greater effect on the mineralisation of phosphorus than of nitrogen. This however requires verification in the case of Taupo pumice. A relatively long-term (3 months) incubation of the 24-year pasture soil was carried out using the periodically aerated mason-jar technique of Thompson and Black (1949). The pH was raised with NaOH or $Ca(OH)_2$. These treatments increased CO_2 respired to the end of the experiment but no consistent effect on nitrogen mineralised (inorganic nitrogen was measured), organic phosphorus mineralised (organic phosphorus and inorganic phosphorus extracted by Truog's extractant were measured) was found. Aeration had been inadequate and denitrification had undoubtedly taken place, explaining the nitrogen results. Inorganic phosphorus however is not lost in this maner and the failure to measure a decrease in organic phosphorus or an increase in Truog available phosphorus was unexpected. It is perhaps worthy of note that in Thompson and Black's work aeration was better in their Exp. 1 than in Exp. 2 and in the latter experiment the mineralisation of nitrogen and phosphorus was reduced relative to Exp. 1. Further, in the case of their virgin soil, with presumably the greater oxygen demand, the relative decrease in mineralisation was greater in the case of phosphorus than in the case of nitrogen. Thus the failure to observe mineralisation of organic phosphorus may have been due to the faulty technique used and the question remains unanswered.

The liming of pumice land presents two difficulties. Exp. 6 indicates that at quite moderately elevated pH values (6.3) a minor-element deficiency can be induced. No attempt to determine which element(s) was involved has been made. Secondly, Fieldes (pers. comm.) has shown that the isoelectric point of the Taupo colloid is about 5.5, the same pH as is commonly found in the field. As the pH is raised, so the nett negative charge rises markedly. If samples of soil at high pH values are allowed to stand, the pH value tends to move back towards the isoelectric point. Fieldes has postulated that the increase in negative variable charge is due to tetrahedral aluminium which co-ordinates hydroxyl groups increasingly as the pH is raised thus increasing the charge. The reversion towards the isoelectric point is due to the equilibration of less accessible sites. The colloid is in effect highly buffered. In practice this will mean that heavy dressing of lime will be needed to obtain even moderate increases in pH especially when the organic-matter level is high.

As against these difficulties an advantage of these soils from the point of view of hastening the mineralisation of organic nitrogen and phosphorus is that only the top inch or two of the soil is of importance. Jackman (1951) has described the profile distribution of organic phosphorus in these soils. Biological fixation of phosphorus, the nutrient commonly in shortest supply, is essentially limited to the top four inches and it is possible that most of the fixed phosphorus is even closer to the surface than his results indicate. Consequently if a liming technique were developed it need be applied only to a shallow layer of soil.

A corollary to these considerations is that, from the point of view of nutrient supply, these soils should not be ploughed so as to bury the

organically rich topsoil. The organic layer is then inaccessible to liming, unless the whole plough layer is limed, and in addition the new topsoil is low in organic constituents and over the years biological fixation of applied phosphorus will occur in this topsoil until the equilibrium level is again reached.

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THE INSECT COMPLEX OF SOWN PASTURES IN THE NORTH ISLAND

IX. THE LEPIDOPTERA AS REVEALED BY SUMMER SWEEP-SAMPLING

By R. A. Cumber, Entomology Division, Department of Scientific and Industrial Research, Palmerston North

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Summary

Some 20 species of Lepidoptera were taken from the main pasture types occurring in the North Island in a daylight sweep-sampling survey carried out during the summer of 1957. The identity of the majority of the species, their distribution, abundance and possible economic importance are discussed.

Introduction

During the summer of 1957, a survey of insects associated with pastures in the North Island was carried out. In the first paper of the series (Cumber 1958) techniques, coverage, and relevant sampling-station details are given, and the insect complex generally as shown by sweep-sampling is analysed and discussed. Subsequent papers have provided information in respect of the Hemiptera, Coleoptera, Hymenoptera, Psocoptera and Neuroptera, Thysanoptera, Orthoptera (Cumber 1959 a, b, c, d, e, f), and Diptera (Cumber and Harrison 1959).

The present account discusses the Lepidoptera with regard to the identity of the species, their distribution, numbers encountered, and possible economic importance.

The survey involves 113 paddock and 108 adjacent roadside samples each comprising a single daylight sweep from an area of approximately 8 square yards.

Distribution maps for 5 species are given, a black dot indicating the presence of a species in contrast to a ring where sampling failed to reveal it.

Results

Suborder **Ditrysia**

Fam. Coleophoridae

These small narrow-winged moths may usually be recognised by their habit of holding the antennae in a porrect position during repose. The larvae are usually miners during their earliest stages, afterwards constructing and inhabiting portable cases which they attach to a leaf or seed-vessel (Imms 1957).

In New Zealand there are two species belonging to the more extensive genus *Coleophora*. One has been positively identified, but the status of the other is still in doubt. The first record of this genus in

the country is that of Lindsay (1927). Information on the biology and distribution of the species and references to the relevant literature are contained in the papers of Hamilton (1944), Cumber (1951), Dumbleton (1952), Stuart (1958), and Kelsey (1958). The New Zealand hosts are white clover (*Trifolium repens* L.), strawberry clover (*T. fragiferum* L.) and red clover (*T. pratense* L.). White-clover seed yields are at times very seriously affected by these species. Both species fly actively at night, so that daylight sweep-netting is a relatively inefficient method of detecting their presence.

Le. 1. *Coleophora alcyonipennella?* Kollar

Distribution

Dumbleton (1952) records (as *C. frischella* L.) this species which apparently also occurs in Europe, Australia, and Tasmania, from Timaru, Ashburton, Blenheim, Nelson, Hastings, and Foxton, and indicates that "it probably occurs throughout New Zealand." In the present survey it was taken in 22 paddock (75 specimens) and 19 roadside (46) samples, from widely scattered localities in the North Island (Fig. 1). The greatest catch (15) was in a roadside sample from Horotiu taken on 23.1.57.

Habits and Economic Significance

See above references. Light trapping (Cumber 1951) produced April with peak flights in January and February. In Ashburton County damaged seed in white and red clover crops of the order of 63% and 8% respectively has been reported.

Le. 20. *Coleophora spissicornis* Haw.

Distribution

Dumbleton (1952) records this cosmopolitan species, which is easily distinguished from the preceding by its banded distal portion of the antennae, from Ashburton, Governor's Bay, Blenheim, Nelson, Foxton, and Hastings, and considers that "it probably occurs throughout the country." In the present survey, single adults were taken in paddock samples from Okere 4.3.57, and Kohinui 28.3.57, and in a roadside sample from Taueru 25.3.57.

Habits and Economic Significance

See above references. Light trapping (Cumber 1951) produced adults only in November and December. Stuart (1958) records a "few stragglers may be seen later than January." The present records show a further extension of the adult flight season, and possibly indicate that as in *C. alcyonipennella?* there is more than one brood each season.

Fam. TINAEIDAE

This very extensive family of relatively small moths includes many pests of stored products. The larvae are known to feed on a great variety of animal and vegetable products.

Fig. 1

Fig. 2

Fig. 1.—Distribution map for the coleophorid *Coleophora alyconipennella?* Le. 1
Fig. 2.—Distribution map for the tinaeid Undetermined. Le. 2.

Le. 2. Undetermined*

This tiny species was taken in 26 paddock (50 specimens) and 21 roadside (98) samples, from widely scattered North Island districts (Fig. 2). The greatest catch (13) was taken in a roadside sample from Te Awamutu on 23.1.57. The habits of the species are not known. It was taken in January (24 samples), February (8), March (14), and early April (1).

Le. 6. Undetermined

A single specimen was taken in a roadside sample from Sanson on 7.2.57.

Le. 8. Undetermined

This species was taken in paddock samples from Dargaville 22.1.57 (1 specimen), Pakipaki 11.2.57 (2), and in roadside samples from Kaitaia 16.1.57 (1), Kai Iwi 24.1.57 (1), and Greytown 25.3.57 (1).

Le 9. *Choreutis bjerkandrella* Thunb.

Distribution

Hudson (1928) indicates that this cosmopolitan species "Although confined to very restricted spots this dark, silver-spotted little insect has

* Undetermined species are held under the numbers indicated in the collections of the Entomology Division substation, Palmerston North.

occurred in many localities both in the North Island and northern part of the South Island." In the present survey it was taken in 4 paddock (4 specimens) and 8 roadside (18) samples, at widely separated localities south of Auckland (Fig. 3), the greatest catch (8) being from a roadside sample taken at Maraekakaho on 11.2.57.

Habits and Economic Significance

The larva feeds on thistles and other composites. Adults fly during the day or at night. It is obviously a casual associate of pastures and not of any importance apart from the slight beneficial effects associated with its attacks on weeds. It was taken in January (1 sample), February (2), March (8), and early April (1).

Fig. 3.—Distribution map for the tinaeid *Choreutis bjerkandrella*. Le. 9.

Le. 10. Undetermined

This species was taken in paddock samples from Kaitaia 16.1.57 (1 specimen) and Whakatane 14.2.57 (1), and in roadside samples from Tolaga Bay 18.2.57 (1), Masterton 25.3.57 (1), Norsewood 26.3.57 (4), and Hunterville 27.3.57 (2). More than one species may be involved here.

Le. 12. Undetermined

A single specimen was taken in a roadside sample from Te Matai on 4.3.57.

Le. 15. Undetermined

A single specimen was taken in a roadside sample from Paekakariki on 31.1.57.

Le. 21. Undetermined

This species (two may be involved) was taken in paddock samples from Warkworth 22.1.57 (1 specimen) and Opotiki 14.2.57 (1), and in roadside samples from Whananaki 22.1.57 (1), Kai Iwi 24.1.57 (1), Mapiu 8.3.57 (1), and Masterton 25.3.57 (2).

Fam. Tortricidae

This family includes many species which are injurious to leaves and stems of dicotyledonous plants.

Le 7. *Tortrix leucaniana* (Walk.)

Distribution

Hudson (1928) states "This little species is common and generally distributed throughout the country." In the present survey it was taken in paddock samples from Tolaga Bay 12.2.57 (1 specimen), Otorohanga 7.3.57 (1), and Mangapakeha 25.3.57 (1), and in roadside samples from Eltham 24.1.57 (1), Whangaehu 30.1.57 (1), Mangaweka 18.3.57 (3), and Kohinui 28.3.57 (1).

Habits and Economic Significance

Hudson further states that "It frequents grassy places, where it is often common, and is usually observed flying close to the ground in the evening." Cumber (1951) in light-trap flight records from Foxton (Paiaka) records this species from October to May with a peak in January. Nothing is known of the larval habits so its importance cannot be gauged without closer studies.

Fam. Crambidae

This family includes the so-called grass moths, which are often very abundant in our pastures. Adults are normally active during the night hours. Larvae feed for the most part, in silken galleries on "grasses, reeds and allied plants, or on mosses" (Imms 1957).

Le. 4. *Crambus flexuosellus* Dbld.

Distribution

According to Hudson (1928) "Except in the north this species is extremely common and generally distributed throughout the country." In the present survey it was taken in 8 paddock (8 specimens) and 17 roadside (29) samples, the greatest catch (8) being in a roadside sample from Mangaweka (18.3.57). The distribution (Fig. 4) agrees with that indicated by Hudson, no specimens being taken north of Auckland.

Habits and Economic Significance

Hudson indicates that adults are present from November to April. Cumber (1951) in light-trap flight records from Foxton (Paiaka), shows that moths are present in every month of the year with considerable numbers from October to June and a peak in March. This is certainly one of the most common night-flying moths as will be evident from the examination of almost any car radiator. Its habits have not been studied, and it may yet prove to be damaging to pastures in some

localities. It was taken in January (4 samples), February (3), March (16), and early April (2).

Le. 16. *Crambus simplex* (Butler)

Distribution

Hudson (1928) states "This rather faintly-marked species has occurred at Napier, Waipukurau, Waiouru, and Wellington in the North Island, and seems to be common and generally distributed throughout the South Island." In the present survey it was taken in paddock samples from Norsewood 11.2.57 (5 specimens) and Matawai 13.2.57 (1), and in roadside samples from Te Pohue 15.2.57 (1), and Masterton 25.3.57 (1).

Habits and Economic Significance

Hudson gives an account of the life history and habits of this species which is commonly associated with tussocks (*Poa* spp.). Adults are present from November to March.

Le. 14. *Scoparia submarginalis?* (Walk.)

A single specimen was taken in a roadside sample from Levin on 31.1.57. Hudson (1928) and Cumber (1951) provide information on this species, which is unlikely to be of importance in pastures.

Fig. 4

Fig. 5

Fig. 4.—Distribution map for the crambid *Crambus flexuosellus*. Le. 4.

Fig. 5.—Distribution map for the lycaenid *Lycaena labradus*. Le. 3.

Fam. Pterophoridae (Plume Moths)

The larvae of plume moths are often associated with composites, feeding on flowers, leaves, and stems. They are not important in agriculture.

Le. 18. *Platyptilia falcatalis* Walk.

A single specimen was taken in a roadside sample from Mapiu on 8.3.57.

Le. 19. *Alucita monospilalis* (Walk.)

A single specimen was taken on 25.3.57 in a roadside sample from Masterton.

Fam. Lycaenidae (Blues, Coppers)

Larval habits in this family are very varied indeed, some species being carnivorous. The family is not of importance in agriculture.

Le. 3. *Lycaena labradus* (Godt.)

Distribution

This species occurs throughout New Zealand and in Tasmania, Australia, Timor, Celebes, India, and many South Pacific islands. In the present survey it was taken in 11 paddock (18 specimens) and 17 roadside (38) samples, from widely scattered North Island localities (Fig. 5).

Habits and Economic Significant

Little is known of the life history of this species in New Zealand. It is often very abundant on dry waste land especially in northern areas, being on the wing from early October to April. It is not important in agriculture.

Fam. Pieridae (Whites, etc.)

The family includes some of the commonest butterflies. Some such as *Pieris* spp. are extremely injurious to cruciferous vegetables and crops. The larvae of other genera feed on Leguminosae and Capparidaceae.

Le. 17. *Pieris rapae* (L.) (Small White)

Distribution

This is the well known white butterfly which accidentally reached this country some 30 years ago and has since proved very troublesome to crucifers generally. In the present survey a single adult was taken in a paddock sample from Whitianga on 6.3.57. The insect is present throughout the country.

Habits and Economic Significance

The habits of the species are well known, there being some 25 references in the New Zealand literature. It is commonly seen in moderate numbers over pasture land, but here its association is a casual one, the adults seeking nectar-producing flowers or the occasional cruciferous weed.

Fam. GEOMETRIDAE (Carpets, Waves, Loopers, etc.)

This very extensive family includes pests of shrubs and trees. In New Zealand, one endemic species occasionally causes defoliation outbreaks in exotic pine plantations.

Le. 5. *Xanthorhoe aegrota?* (Butl.)

Distribution

The identity of the species is not certain. Hudson (1928) records the species from the Wellington province southwards. In the present survey it was taken in paddock samples from Opotiki 14.2.57 (2 specimens), and Porangahau 26.3.57 (4), and in roadside samples from Gisborne-Tiniroto 12.2.57 (1), Tolaga Bay 12.2.57 (1), Taupo-Rotorua 4.3.57 (1), Taueru 25.3.57 (1), Dannevirke 26.3.57 (1), and Manaia (Taranaki) 3.4.57 (1).

Habits and Economic Significance

According to Hudson, the moth is on the wing from November to March and frequents open situations in the vicinity of forests. This species was often seen in moderate numbers where poorer-class rank pastures were sampled. It is probably not of economic importance in pastures generally.

Fam. NOCTUIDAE (Cutworms, Army Worms, etc.)

This extremely large family contains many injurious foliage feeders, and is at times of considerable economic importance in pastures. The great majority of species fly at night, remaining well concealed during daylight hours. Daylight sweep-netting is thus not a very efficient method of detecting their presence.

Le. 11. *Pseudaletia separata* (Walk.) (Introduced Army Worm)

Distribution

This well known cosmopolitan species is widely distributed in the country. In the present survey single specimens were taken in roadside samples from Waikohu 13.2.57 and Te Matai 4.3.57.

Habits and Economic Significance

This nocturnal species is apparently the one responsible for serious local army-worm outbreaks in pastures and cereal crops in the North Island.

Le. 13. *Heliothis armigera* Hubn. (Tomato Fruitworm)

Distribution

This cosmopolitan species is of widespread occurrence in New Zealand. In the present survey a single adult was taken in a roadside sample from Maramarua on 6.3.57.

Habits and Economic Significance

This day-flying species is on the wing from January to March. The larvae may attack a wide variety of garden plants (Atkinson *et al.* 1956; Hudson 1928). This insect shows marked fluctuations in

numbers from year to year depending upon the varying seasonal conditions. It is known to be parasitised by a number of hymenopterous insects, these emerging from the overwintering pupae. It is not of importance in pastures.

Discussion

The majority of Lepidoptera are night-flying insects, and for this reason daylight sweep-netting is unlikely to give an accurate indication of their numerical representation. Sweep-netting is rather damaging to the smaller species especially, so that identifications are often extremely difficult in such families as the Tinaeidae. Sweep-netting at night, if it were possible, would probably provide quite a lot of additional useful information, but it should be remembered that many species restrict their flights to certain rather specific weather conditions, often quite unsuited to sweep-netting, and different species may fly at different times during the hours of darkness. The assessment of the relative importance of the species encountered in this survey is a very difficult task.

There is already adequate information on the importance of *Coleophora* spp. in clover seed production. Some of the tinaeids present may be of minor importance. It would appear desirable to have further information on the habits of certain *Crambus* spp., especially *C. flexuosellus* which may prove to be of more importance in some areas than has hitherto been suspected. The noctuid *Pseudaletia separata* although poorly represented in the present survey is known to be of considerable importance, apparently being the species mainly concerned in the North Island army-worm outbreaks. *Oxycanus* spp. were not taken during sweep-netting although known to be present in moderate numbers in some North Island pasture areas.

In pastures that are moderately grazed, larval Lepidoptera unless occupying ground tunnels are vulnerable to trampling. Under conditions of lighter grazing and increased roughage and ground litter, increased populations are to be expected.

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PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

XX. THE EFFECT OF EXTRACTS CONTAINING THE FACIAL-ECZEMA TOXIN ON CELLS IN ARTIFICIAL CULTURE

By A. M. Murphy,* Central Laboratory, Public Hospital, Auckland, and N. A. Worker, Ruakura Animal Research Station, Department of Agriculture, Hamilton

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Summary

The effect of extracts of toxic and non-toxic grass on a range of cells growing in artificial culture has been investigated.

HeLa cells and human amniotic cells growing in serial culture were found to be more sensitive to the effects of toxic extracts than to non-toxic extracts. Human amniotic cells in primary culture and guinea pig kidney cells in primary culture were apparently unaffected by either.

HeLa cells were shown to be affected also by crude ether extracts of cultures of the fungus *Sporidesmium bakeri* Syd. Ether extracts of fungus partially purified by fractionation and containing only one-fortieth part of their original weight were likewise shown to be still highly active.

There was a high correlation between toxicity established by guinea pig feeding and by HeLa cell tests.

It is suggested that the facial-eczema toxin is responsible for producing the effects observed in HeLa cell cultures.

Introduction

Damage to bile-duct epithelium is generally regarded as the essential primary lesion in facial-eczema liver damage in the sheep, guinea pig, and rabbit (Cunningham *et al.* 1942; Evans *et al.* 1957; McFarlane *et al.* 1959; Clare 1959). There is little convincing evidence to date to suggest that structures other than bile-duct epithelium, such as parenchymal cells or vascular tissue, are affected in the initial stages of the disease in grazing animals or in animals fed toxic grass or crude fungal cultures.

Recently, however, studies undertaken at the Ruakura Animal Research Station to compare the relative effectiveness of toxic extracts administered by the oral and parenteral routes have demonstrated, in certain cases, the presence of both vascular and hepatic cell damage. The most consistent effects were produced by direct intraportal injection of high levels of toxic extract. These findings, which will be discussed in more detail in a subsequent communication (Worker and Dodd in preparation), suggested the possibility that, providing a sufficiently high concentration of toxin is present, cells other than bile-duct epithelial cells might be affected by the facial-eczema toxin. It

* Present address: The Institute of Clinical Pathology and Medical Research, Lidcombe, New South Wales.

was decided, therefore, to investigate the possible effects that extracts of toxic grass and of toxic cultures of the fungus *Sporidesmium bakeri* Syd. might have on a range of cells growing *in vitro*. This communication presents the preliminary results of these studies.

Experimental

Preparation of Extracts

Extracts of toxic and non-toxic grass were fractionated to the "3000" and "70,000" stages using the technique described by White (1958). (The terms "toxic" and "non-toxic" refer to the toxicity of the grass as measured by guinea pig feeding trials carried out by J. C. Percival at Ruakura; see, for example, Percival, 1959). All toxic grass samples used in the present experiments were of at least moderate toxicity (see Perrin, 1957). Ether extracts of fungal cultures and potato-carrot fungal culture medium were prepared by the method of White (1959).

Preparation of Cell Cultures

HeLa cells. A culture of these cells was obtained from W. F. Scherer in 1954 (see Scherer *et al.* 1953). Until recently they have been cultivated in flat bottles in a liquid nutrient medium of the following percentage composition: human serum, 10.0; lactalbumin hydrolysate, 0.5; yeast extract, 0.5; Hank's balanced salt solution, 89.0. Lately, owing to difficulties in obtaining a continuous supply of human serum, they have been cultured in a substitute medium (see below) containing calf serum. Before use the cells were transferred to test tubes, the volume of the cultures being adjusted beforehand so that each tube contained approximately 250,000 cells in 0.9 ml medium.

Human amniotic cells in serial culture. Human amniotic cells were obtained in serial culture in the Auckland Hospital Central Laboratory and were maintained in a manner similar to HeLa cells. During adaptation to serial cultivation the cells underwent "transformation" and were morphologically similar to HeLa cells. They were put up in test tubes, 250,000 cells being contained in 0.9 ml medium.

Human amniotic cells in primary culture. A suspension of human amniotic cells was obtained by treatment of amniotic membranes with 0.25% trypsin for two hours at 37°C. The cells were centrifuged and suspended in culture medium (see below), and test-tube cultures were prepared by the introduction of 0.9 ml of the suspension (containing approximately 500,000 cells) into each tube. After five to seven days incubation, the cells had multiplied to form a confluent monolayer and were ready for use. (For further details of the procedure see Zitcer *et al.* 1955).

Guinea pig kidney cells in primary culture. Guinea pig kidney cells were prepared in a manner similar to amniotic cells. Kidneys from suckling guinea pigs less than one week old were used.

Culture Medium

At the time of the tests all cells were maintained in a fluid culture medium of the following percentage composition: calf serum, 5.0;

lactalbumin hydrolysate, 0.5; yeast extract, 0.5; Hank's balanced salt solution, 94.0.

Introduction of Toxic Extracts

Extracts were dissolved in a small volume of absolute ethanol and serial dilutions were made in tissue-culture nutrient medium. Previous experiments had shown that the concentration of alcohol in the final dilutions had no effect on the cell cultures. From each dilution 0.1 ml was added to a cell culture bringing the total culture volume to 1.0 ml. Each dilution was assayed in duplicate.

Assessment of Toxicity

Cultures were examined daily for four days for evidence of cell degeneration. Degenerative changes did not take place immediately but progressed slowly, reaching a maximum in approximately 72 hours (Table 1). End points were taken as the highest dilution which caused the great majority of the cells in the culture to degenerate.

Results and Discussion

The results in the present series of experiments are shown in Tables 1 to 4. From Table 1 it will be seen that, of the cell cultures tested, only HeLa cells and human amniotic cells in continuous culture were sensitive to the effects of toxic extract. Maximum effects were recorded at three days. Guinea pig kidney cells and human amniotic cells in primary culture, at least over the range of concentrations investigated in the present studies, were not affected. It should be emphasised that cultures of the latter two cells were tested in a single experiment only. It is possible that their apparent resistance was due to a larger number of cells present in these cultures than in the corresponding HeLa cultures. There was no other obvious explanation of this difference and no further investigation was carried out. On the basis of these results, and also in view of the relative sensitivity of HeLa cultures and the ease with which they could be handled, it was decided that these cells should be used in subsequent work.

TABLE 1. Effect of 70,000 Extracts of Toxic Grass (ex Gisborne) on Cells in Artificial Culture

Dose 90 μg per tube (cf. Table 2)

Culture	Evidence of Degeneration			
	1 day	2 days	3 days	4 days
1. HeLa cells	negative	positive?	positive	highly positive
2. Human amniotic cells in continuous culture	negative	negative	positive	positive
3. Human amniotic cells in primary culture	negative	negative	negative	negative
4. Guinea pig kidney cells in primary culture	negative	negative	negative	negative

TABLE 2. EFFECT OF GRASS EXTRACTS ON HeLa CELLS

Exp.	Specimen	Wt. of Extract (mg)	Smallest Amount of Extract Causing Degeneration (μg)	Lowest Concentration Causing Degeneration (μg/mg)	Ratio Non-toxic/ Toxic
1	3000 extract toxic grass (Gisborne)	616	156	0.25	3.58
	3000 extract non-toxic grass (lawns, RARS, summer)	663	600	0.91	
	70,000 extract toxic grass (Gisborne)	89.5	45	0.50	9.68
	70,000 extract non-toxic grass (lawns, RARS, summer)	30.8	150	4.87	
2	70,000 extracts toxic grass				
	Gisborne	89.5	45	0.50	Mean value 12.0
	Fertility Centre, RARS	51.8	21	0.41	
	Composite*	13.8	6.1	0.44	
	70,000 extracts non-toxic grass				
	Gisborne (autumn)	28.8	57	2.0	
	Sheep farm, RARS (autumn)	18.7	93	5.0	
	Sheep farm, RARS (spring)	9.4	94	10.0	
	Dairy farm, RARS (spring)	12.3	61	5.0	
	Lawns, RARS (summer)	30.8	150	5.0	

* Grass, from several sources, bulked for chemical work by E. P. White.

TABLE 3. EFFECT OF FUNGAL CULTURES AND CRUDE ETHER EXTRACTS OF CULTURES ON HeLa CELLS

Specimen	Weight of Extract (mg)	Highest Dilution Causing Degeneration	Smallest Amount Causing Degeneration (μg)
Potato-carrot medium (unextracted)	—	Non-toxic undiluted	—
Fungal macerate; P.C.7 + P.C. 11	—	1/16	approx. 100 mg diluted culture
Potato-carrot medium extract (≡ 100 ml)	3.6	1/10	36.0
1. P.C. 7 RARS (≡ 100 ml)	11.5	1/400	2.88
2. P.C. 10 "C": S.B. (≡ 100 ml)	9.7	1/200	4.85
3. C.A.A. "C": S.B. (≡ 100 ml)	9.0	1/400	2.25
4. P.C. 5: RARS (≡ 100 ml)	8.8	1/400	2.20

TABLE 4. Effect of Concentrated Fungal Extracts on HeLa Cells

Specimen	Guinea Pig Test Gradings* at Three Levels of Intake (mg.)			Weight of Extract (mg.)	Highest Dilution Causing Degeneration	Smallest Amount Causing Degeneration (μg)
1 PC 5/5	0.36 mild-mod.	1.16, moderate	—	0.860	1/3200	0.027
2 PC 5/8	0.26, moderate	0.54, v. mild	1.30, severe	0.934	1/6400	0.015
3 PC 7/9	0.79, normal	1.58, normal	3.80, mild	0.718	1/400	0.177
4 PC 5/10	0.42, mild	0.84, mild	2.1, moderate (22 days only)	0.640	1/400	0.160
5 PC 7/11 (i)	0.51, moderate	1.02, moderate-mod. severe	2.55, mild (21 days)	0.594	1/400	0.148
PC 7/11 (ii)				0.980	1/800	0.114
6 SB 23/2	0.56, normal	1.73, mild-mod.	2.73, mod. severe	0.907	1/200	0.453
7 RLMS XX116F	0.34, mild	—	—	0.340	1/400	0.085

* See Perrin (1957)

Table 2 summarises work carried out with grass extracts. The results of the preliminary trials with these extracts (Table 2, Exp. 1) indicated that, on a weight basis, extracts of toxic grass concentrated to the 3000 stage were over three times as effective when added to HeLa cultures as extracts of non-toxic grass, while at the 70,000 stage extracts of toxic grass were almost ten times as effective. This latter finding was also confirmed by the results of a further experiment (Table 2, Exp. 2) in which the activities of three toxic and five non-toxic extracts, all processed to the 70,000 stage, were compared. With the exception of the Gisborne non-toxic grass, there was at least a ten-fold difference in toxicity between extracts of toxic and non-toxic grass. This particular Gisborne non-toxic sample was collected during a period when facial eczema was prevalent and it is of interest to note that subsequently it was shown by N. T. Clare (pers. comm.) to contain a spore load in excess of 20,000 per gramme. Other non-toxic samples had negligible spore loads, while the three toxic samples tested were shown to contain between 200,000 and 400,000 spores per gramme.

Although at the time these initial trials were carried out methods were available for preparing 200,000–400,000 concentrates (White 1959), no further experiments with grass extracts were undertaken, as this work coincided with the discovery of the fungal origin of the facial-eczema toxin (Thornton and Percival 1959), and attention was thereafter diverted to the testing of pure fungal cultures and fungal extracts.

The results of trials involving macerated whole fungal cultures and crude ether extracts of these are presented in Table 3. Potato-carrot medium was not toxic to HeLa cultures in the highest concentration used, which was 10% of the culture medium (i.e. 0.9 ml nutrient culture medium + 0.1 ml potato-carrot medium). On the other hand a macerate of 7-day fungal growth plus potato-carrot medium, which was known from previous guinea pig evidence to contain facial-eczema toxin, was toxic when incorporated in this proportion after 16-fold dilution. Crude ether extract of potato-carrot medium was shown to have an effect when incorporated at a level of 36 μg. In contrast, extracts of four different fungal cultures of proven toxicity to the guinea pig were effective against the HeLa at levels of 2.88, 4.85, 2.25, and 2.20 μg, respectively.

Table 4 summarises results obtained using concentrated ether extracts of fungus. Six different concentrates prepared by E. P. White were used along with one further concentrate prepared by R. L. M. Synge. The concentrates prepared by White were eluates from acetic-acid-treated alumina (White 1959; Synge and White in press) and contained, on the average, about one-fortieth part by weight of the original crude fungal ether extract. That prepared by Synge was a chromatographic subfraction from PC 5/5 obtained during an attempt at further concentration of the toxin in this sample. The presence of toxicity in these extracts had been established previously by White using the guinea pig assay (see table). From the results obtained with the HeLa it is clear that, even after a considerable bulk of the weight of the original ether extracts had been removed, the remaining material

was still highly active. It is particularly noteworthy that the amount of extract required to produce an effect was, in each case, only a fraction of a microgramme.

The above observations clearly demonstrate that HeLa cells in artificial culture are particularly sensitive to the effects of extracts of toxic grass and of the fungus *Sporidesmium bakeri* Syd., the agent responsible for the production of the facial-eczema toxin (Thornton and Percival 1959). This activity has also been shown to be present in the most highly concentrated extracts of the fungus that have been prepared to date.

The evidence obtained in the foregoing experiments does not directly indict the facial-eczema toxin as being the factor responsible for cell degeneration. The possibility that some factor other than the toxin is involved cannot be overlooked. The fact, however, that high activity is still found in the best toxic fungal concentrates available, containing approximately only one-fortieth part of the dry weight of the original crude ether extract, does strongly suggest the possibility that the toxin is involved.

Experiments are at present planned at Ruakura to confirm and extend the above observations, using more highly concentrated fungal extracts when these become available. The practicability of a micro-assay based on these findings is also currently under consideration. The obvious advantages of such a technique, should it prove suitable, in terms of time and materials, need not be emphasised.

Acknowledgments

The authors wish to record their thanks for the assistance received from Mr A. Fyvie and Mr H. de Langen in the preparation of extracts.

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BLOAT IN CATTLE

XVII. A TECHNIQUE FOR RECORDING PRESSURE CHANGES IN THE RETICULO-RUMEN OF CATTLE, USING SMALL ELECTRICAL PRESSURE TRANSDUCERS AND A FOUR-CHANNEL RECORDER

By C. S. W. Reid,[1] A. W. Melville[2] and J. B. Cornwall[2]

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Summary

A small pressure transducer of the differential transformer type and a stable, sensitive, carrier-amplifier for use with it are described. An apparatus has been constructed consisting of four such units, together with their power-supply, the output being displayed by a modified Sanborn 4-channel heated-stylus recorder.

Techniques are given for registering pressures in different regions of the reticulo-rumen of cattle having rumen fistulae. The transducers are located internally at the points from which the recording is being made, thus avoiding the errors inherent in external measurement through pressure-conducting systems.

The equipment is versatile and can be used to record any physiological variable which may be expressed as a change of pressure, resistance, or displacement. Examples of such applications are given, while the Appendix describes a suitable pressure chamber for use with the transducer.

Introduction

The classical method of recording pressures in the gastro-intestinal tract involves the use of a lightly-inflated, thin-walled rubber balloon inserted into the rumen and connected to a water manometer or a Marey tambour writing on a kymograph. It has long been used in studies of the ruminant stomach, e.g. Toussaint (1875), Wester (1926), Schalk and Amadon (1928), Phillipson (1939), Balch *et al.* (1951). Although it possesses the merit of simplicity, the technique suffers from a number of deficiencies, the most important being the high inertia and high volume/pressure coefficients of such systems. The dynamic properties are inadequate for faithful registration of the fastest pressure fluctuations and large errors of amplitude and timing may result (see Quigley and Brody 1950; Code *et al.* 1952, 1954; Rowlands *et al.* 1953; Lorber and Shay 1954, Texter *et al.* 1957), particularly if "resistance" manometers are included in the conducting line (e.g. Quinn *et al.* 1938). A further disadvantage when recording from the reticulo-rumen is that the inherent instability of the system is increased by diffusion of gas from the stomach contents into the balloon, which may result in a rapid rise of the baseline (Reid and Johns, unpublished results).

[1] Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.

[2] Auckland Industrial Development Laboratories. Department of Scientific and Industrial Research, Auckland.

The established principles for accurate measurement of pressures in the blood system (see Wiggers 1928) were first applied to the study of the alimentary canal by Brody *et al.* (1940). Since then two major techniques, both originally used in circulatory investigations, have been evolved to replace the balloon method. These are:

1. The use of an open-ended tube connected to a sensing device having a low inertia and low volume/pressure coefficent; and
2. The insertion of a miniature pressure-sensitive device directly into the gut.

Brody *et al.* (*loc. cit.*) used an open-ended tube connected to an optical manometer, and improved versions of this apparatus have been described by Brody and Quigley (1951) and Rowlands *et al.* (1953). An alternative method, in which the optical manometer is replaced by an electro-manometer, has been described by Brody and Quigley (1951), Lorber and Shay (1954), Dziuk and Sellers (1955b), and Texter *et al.* (1957), and this has been used in cattle by Sellers and his associates (Dziuk and Sellers 1955a, b; Stevens and Sellers 1956, 1959) and in goats by Bell (1958) and Andersson *et al.* (1958).

The conducting medium and the design of the conducting system are important factors affecting the performance of the open-tube apparatus. Brody and Quigley (1951) preferred gas to liquid as the medium, recommending helium for maximal efficiency. The total volume of a gas-filled system must be as small as possible—that of the equipment described by Quigley and Brody (*loc. cit.*) is less than 1 ml, the conducting leads being of 1 mm internal diameter. Most other workers, e.g. Lorber and Shay (1954), Dziuk and Sellers (1955b), have used saline as the medium. Because of the higher viscosity of liquid, conducting leads of larger bore are required. Careful attention has to be given to "damping" the system to control resonance and reduce artefacts due to movement of the liquid-filled tube. Correction for the hydrostatic effect of differences in level between tube tip and manometer is necessary, which may be difficult as the differences are often not precisely known and may vary during contractions of the viscus. Whether gas or liquid is employed, a means of maintaining a slow stream of medium into the system is usually included to reduce clogging of the mouth of the tube with food residues.

The technique of introducing a pressure-sensitive device directly into the gut has the distinct advantage of eliminating conducting systems and their drawbacks. The sensing device commonly used is an electronic transducer of the inductive (differential-transformer) type, an instrument also originally developed for registration of blood pressures (Wetterer 1943; Gauer and Gienapp 1950), and methods have been described by Brody and Quigley (1951) and Code *et al.* 1954). The main disadvantage of this technique is that once the transducer has been placed in position, its performance cannot easily be checked again until the end of recording. Consequently a high degree of stability is demanded of both transducer and its ancillary equipment.

The present paper describes an apparatus for recording pressures in the reticulo-rumen of cattle by the internal transducer technique. A robust inductive transducer of improved performance and stability has

been developed and an amplifier of high stability for use with it. The apparatus consists of four of these transducers and their associated amplifiers, a regulated power supply, and a modified four-channel Sanborn heated-stylus recorder, the output being displayed on heat-sensitive paper. Linear pressure ranges of 0–20, 0–40, 0–80, 0–160 and 0–320 mm Hg are available, so arranged that in each case small negative pressures may be recorded, while a suppressed zero control allows greater sensitivity to be used to record pressures whose variations are small relative to their mean value. There are four solenoid-operated marker pens in addition to the galvanometer pens, and a selection of chart speeds from 0.125 to 25 mm/sec is provided. Methods for locating the transducers within the reticulo-rumen are described. The equipment is versatile and can be used to record any physiological variable which may be expressed as a change of pressure, resistance, or displacement. A pressure chamber suitable for use with the transducer in such applications is described in the Appendix.

Description of the Apparatus

The Pressure Transducers

The pressure-transducers have a movable ferroxcube* core providing variable coupling between a primary and two secondary coils. Dimensions and winding details are shown in Fig. 1. A feature of this design is the rugged double-suspension of the core. The stiffness of the transducer is determined wholly by the co-axial suspension springs; the latex covering sheath (see below) serves only to provide an airtight seal between back and front of the plunger.

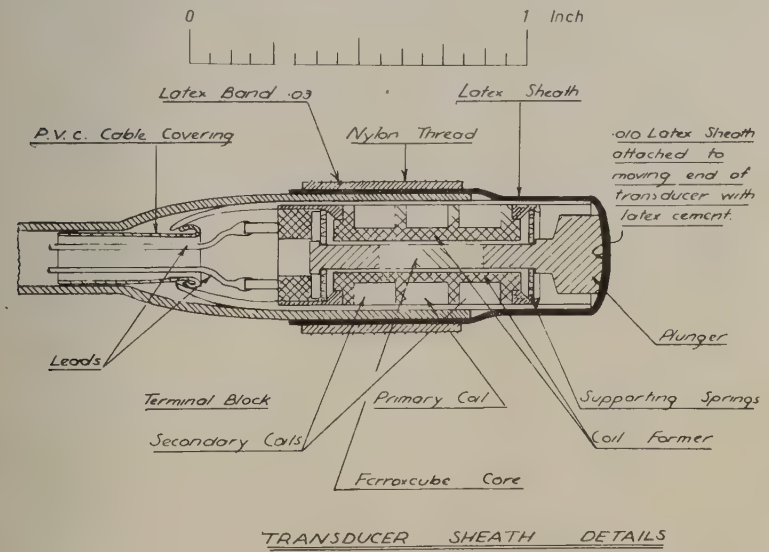

Fig. 1.—Details of the construction of the transducer.

* Supplied by Philips Electrical Industries N.Z. Ltd.

The sensitivity of the transducers is approximately 75 microvolts/mm Hg/volt input. The volume/pressure coefficient is of the order of 3.5×10^{-5} cc/mm Hg. A frequency response curve obtained from a transducer in a liquid-filled chamber (described in the Appendix) is shown in Fig. 14 (curve A).

For convenience, the transducers are set in the ends of cables 3 m long. The cables are formed of medium-soft polyvinyl chloride plastic tubing approximately 6 mm in external diameter, with 1 mm thick walls. The connecting wires run down through the tubing to a 6-pin male HBJ connector, the air gap around the wires ensuring that the back of the transducer diaphragm is at atmospheric pressure.

The Transducer Bridge

The primary winding is energised from an oscillator and amplifier, the input being adjusted to 3 volts at 3,500 cycles per second (see Fig. 2). A high stability resistor in series with the primary helps to "swamp" the effect of the resistance temperature coefficient of the winding.

The two secondaries are connected as adjacent arms of a bridge which is completed by a potentiometer, the "R Balance" control, as shown in Fig. 2. The capacitative balance ("C Balance") is required to correct any inherent phase difference between the two secondary voltages, and to compensate for stray capacity. Part of the latter is due to the capacity between the transducer connecting wire and earth. When the junction of the two secondaries is used as the signal lead with the centre of the "R Balance" earthed, movements of the leads may cause a change of the balance position. Earthing of the transducer connection overcomes this problem by lowering the impedance of the connecting cables to earth and leaving the centre of the potentiometer as the input connection to the amplifiers following.

Once the sensitivity of each transducer has been determined, the calibration of each recording channel may be checked by resistively unbalancing the transducer bridge by the equivalent of a pressure of, say, 40 mm Hg. A switched calibration resistor is included for this purpose. These standardised resistors are made up as plug-in units and they are matched to the individual transducers, so that any transducer may be used with any of the amplifiers.

The suppressed zero ("Strobe") control is a parallel resistive balance, with a range equivalent to 150 mm Hg.

As shown in the final circuit diagram, end resistors are used with these controls to restrict the range and to give smooth operation. The "R Balance" is an 8-turn helipot.

The Amplifier Units

The circuits of the oscillators, amplifiers, and the power supply are shown in Fig. 2.

Phase shift oscillators are used. Each is adjusted independently for maximum undistorted output by variation of the degenerative cathode resistor, the screen voltage having been set for maximum gain. Under

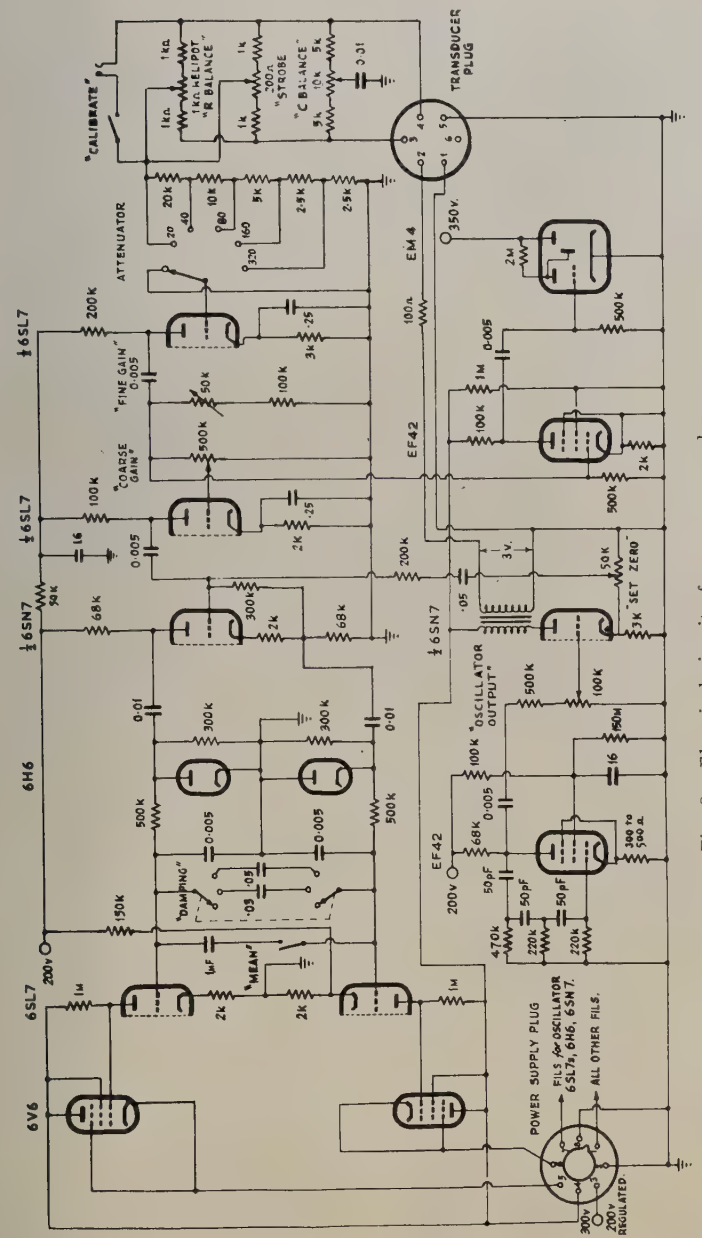

Fig. 2.—Electrical circuit of pressure recorder.

these conditions maximum stability is obtained. The buffer stage is conventionally transformer-coupled to the transducer, and the potentiometer in the grid circuit provides the "Oscillator Output" control. With the output set at 3 volts, approximately 0.8 V is dropped across the swamping resistor.

The output from the transducer bridge circuit is applied to an attenuator providing the full-scale sensitivities of 20, 40, 80, 160 and 320 mm Hg. These ranges are multiples of the linear deflection in mm. A two-stage amplifier follows, with coarse and fine gain controls preceding the second stage. A conventional phase splitter provides a symmetrical voltage to a full-wave demodulator, the output of which is smoothed and applied to a push-pull d.c. coupled driver stage. The 6SL7 driver stage is operating near the limits of linearity so that smoothing of the rectified a.c. is necessary to prevent grid current flowing on the peaks.

The d.c. driver stage which receives positive grid voltage has additional cathode bias derived from the regulated high-tension voltage supply which depresses the static position of the stylus to the bottom of the scale.

The push-pull output tubes operate as cathode followers with the recorder coils connected as the cathode loads, and, providing the driver stage anode loads are matched, linear full-scale deflection is obtained from the d.c. amplifier.

Owing to the non-linearity of the rectifying diodes for very small inputs, accurate balancing of the transducer is difficult using the recorder pen as an indicator. For this reason the signal from the first stage is amplified and presented on a "magic eye" which indicates the null balance very accurately.

A variable amount of "upscaling" is provided so that negative pressures may be recorded. This is achieved by feeding a fraction of the oscillator buffer stage output voltage to the grid of the phase splitter of the amplifier. The phase of this signal is arranged to coincide with that resulting from the application of a positive pressure to the transducer and the amplitude is normally adjusted to provide 20% of the full-scale deflection. Since each record occupies 50 mm of the chart, the zero setting is therefore 10 mm from the bottom of the scale, positive pressures being registered in the remaining 40 mm.

The Power Supply

The power supply is divided into two sections, each supplying two amplifier units. A conventional series regulated supply at 200 V provides the h.t. requirements of all critical stages, and an unregulated voltage of 320 V is supplied for the d.c. amplifier section.

A constant voltage transformer supplies all filament voltages.

The Recorder

The amplifiers and their power supply are mounted in a console unit with a Sanborn four-channel, heated-stylus recorder.

The Sanborn recorder has been modified by the addition of extra marking pens and by alterations to the paper feed.

Besides the normal marker pen at the bottom of the chart, three extra marker pens have been added, mounted between the recording pens. There are thus four marker pens in all, one below each galvanometer record. The power for all the marker pens is supplied internally, while four sockets mounted on the control panel allow leads to signal switches to be plugged in as desired.

A time scale is obtained by connecting one of the pens to a rotary switch driven by a synchronous motor, providing intervals of 1, 5, 10, 30, and 60 seconds.* The built-in Sanborn 1-second time signal is not used.

The Sanborn paper-feed gear box has been modified to give a selection of paper speeds of 0.125, 0.25, 0.5, 1.25, 2.5, 5.0, 12.5 and 25.0 mm/sec. A socket wired in parallel with the paper-feed motor switch allows an extension lead to be plugged in and the motor to be controlled remotely.

Setting-up Procedure

Preparation of the Transducers

The transducers are sealed by first covering with a latex sheath, fitting a heavy latex sleeve over the sheath and then binding with braided nylon thread, which is not readily attacked by rumen micro-organisms. The procedure is shown in Fig. 3.

The sheath has a wall thickness of approximately 0.25 mm and an internal diameter which allows a sliding fit over the exposed metal casing at the end of the unit. The sheath is first turned inside out and cemented to the centre of the transducer plunger with latex cement. When the cement is dry, the sheath is slid backwards on to the unit. To release any drag on the plunger, the sheath is then rolled forwards to the tip of the casing again and allowed to unroll back of its own accord. The annular latex diaphragm now formed around the plunger should be slightly slack.

The sleeve is 2 cm long, with an internal diameter about 7.2 mm and a wall thickness of 0.75 mm. It is placed around the sheath by rolling it up, slipping it over the transducer with the aid of a 3-pronged spreading tool, and unrolling it in position. The binding of surgical braided nylon† is then wound on. Since this thread shrinks slightly in water, the binding tightens on wetting and effectively seals the transducer against all seepage.

To give lateral and end-on protection to the slightly protuberant plunger, a further sleeve of rubber tubing is usually slipped over the transducer. The tubing used is medium-resilience laboratory tubing

* A time clock, type B113, manufactured by C. F. Palmer Ltd., Brixton, London, England.

† "Arbralon" braided nylon thread, size 2 or 3, manufactured by Armour Laboratories, Lindsey St., London, England.

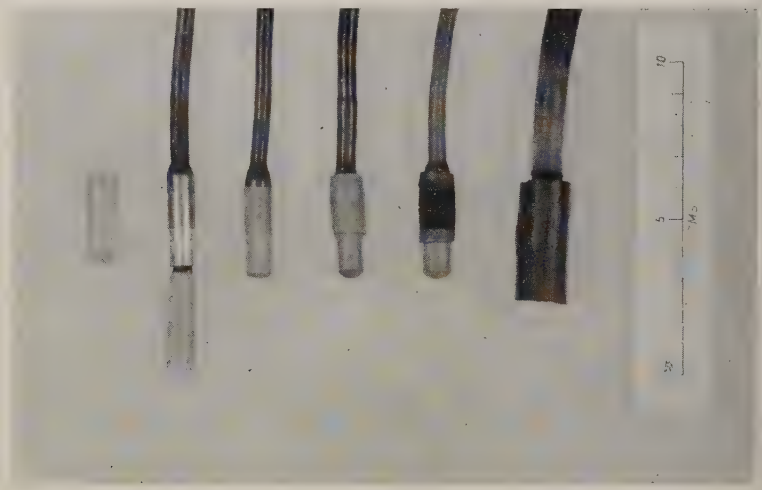

Fig. 3.—Stages in the sealing and protecting of transducers (*l to r*): Thin latex sheath; sheath everted and cemented to plunger of transducer; sheath rolled back into position; latex sleeve in place over sheath; braided nylon binding over sleeve; rubber tubing protecting sleeve over transducer, plastic reinforcing over cable (latter used only when end-weight to be attached).

with a wall thickness of approximately 2.5 mm, and is a push fit over the bound transducer.

When the transducer is to be enclosed in a weight, a sleeve of soft polythene tubing is slipped over the cable to strengthen and protect it. This tubing has an internal diameter of 6.25 mm and a wall thickness of about 2 mm.

Setting Calibration Resistances

Each calibration resistance consists of a fixed resistor and a rheostat wired in parallel across a plug. It is matched to the output of the transducer with which it will be used, as follows:

The transducer is inserted in a suitable pressure chamber connected to a water manometer and submerged in a water bath at the operating temperature, in this case 39°C. The calibration resistance to be set is plugged into the socket on the amplifier panel. The transducer bridge is balanced and the zero set as described below, with the transducer chamber at atmospheric pressure. The "Attenuator" is then switched to the 80 mm Hg full-scale range. A pressure of 40 mm Hg, as shown by the water manometer, is applied to the transducer and the amplifier gain adjusted to give a pen deflection of 20 mm from zero. The pressure is released. The "Calibration" switch is now depressed and the rheostat of the calibration resistance adjusted until the pen deflection is again 20 mm from zero.

Once set in this manner, the calibration resistance need be checked only at intervals. When it is intended to use the transducers at other than the normal operating temperature, the calibration resistances should be reset at the new temperature.

Standardisation of the Amplifiers

The electrical equipment requires a period of 20 min to reach a stable working temperature. During this time the transducers are warmed in a water bath at 39°c if they are to be used in the rumen.

With the transducers at the surface of the bath so that they are at atmospheric pressure, each transducer bridge circuit is balanced for zero output with the "R balance" and "C balance" controls, the "Zero" and "Strobe" controls both being previously turned fully anticlockwise. The attenuator is switched to the earthed position and the "Zero" control adjusted to bring the recording pen 10 mm from the bottom of the scale. When the attenuator is switched to the 20 mm range there should be no movement of the pen. If there is a small movement, it is permissible to correct this by a slight adjustment of the "R balance" control. There should then be no movement of the pen when the attenuator is switched through the ranges.

The attenuator is now switched to the 80 mm range and the calibration resistance belonging to the individual transducer being used is plugged in. The gain control is adjusted so that depression of the "calibrate" switch gives 20 mm deflection from the zero line.

The apparatus is now ready for use.

Recording

To protect the galvanometers from damage while the transducers are being placed in their final recording positions, the amplifiers are heavily damped by switching in the integrating circuit marked "Mean" and the attenuators are switched to the least sensitive range. Once the transducers are in place, the "Mean" switches are opened again, the attenuators set to the desired sensitivity, and recording commenced. Any necessary adjustments to the position of the tracing on the chart are made with the "Strobe" control.

At the conclusion of recording, the galvanometers are protected as before while the transducers are extracted. The transducers are placed immediately in a bucket of water at 39°c and a routine check made of the amplifier gain and of the zero position, taking care to see that the "Strobe" control has been returned fully anticlockwise.

Methods for Recording Pressures at Different Points in the Reticulo-rumen

The cattle used at this laboratory have oval-shaped rumen fistulae about 8 × 12 cm. All parts of the reticulo-rumen may be reached by insertion of an arm. The fistulae are maintained and sealed by two-piece, fabric-reinforced rubber cannulae of the type described by Balch and Johnson (1948).

Reticulum

The standard recording point used in the reticulum is the floor (position 1, Fig. 8).

When recording from this compartment, one of the main difficulties is to prevent displacement of the pressure-sensitive element. Not only must the cable pass over several mobile structures but also a long length is exposed to entanglement with the ingesta, especially during feeding. Two anchor weights are therefore used, the end-weight (1 kg), which encases the transducer, and the cable-weight (0.75 kg), attached 25–30 cm behind the end-weight. The cable-weight, by holding the cable down on the floor of the anterior rumen sac (position 2, Fig. 8) after it has passed over the reticulo-ruminal fold, considerably reduces the risk of tangling.

The weights are made of brass with a heavy plating of chromium. The components of the end-weight are shown in Fig. 4 and of the cable-weight in Fig. 5, while Fig. 6 shows the assembled weights attached to the cable ready for use. It will be seen that the cable-weight is split off-centre, the thicker member containing the cable chamber. The soft rubber locking-block is about 1 mm thicker than the depth of the cable chamber. When the second half of the weight is screwed down, therefore, the block expands radially, and in so doing firmly grips the cable.

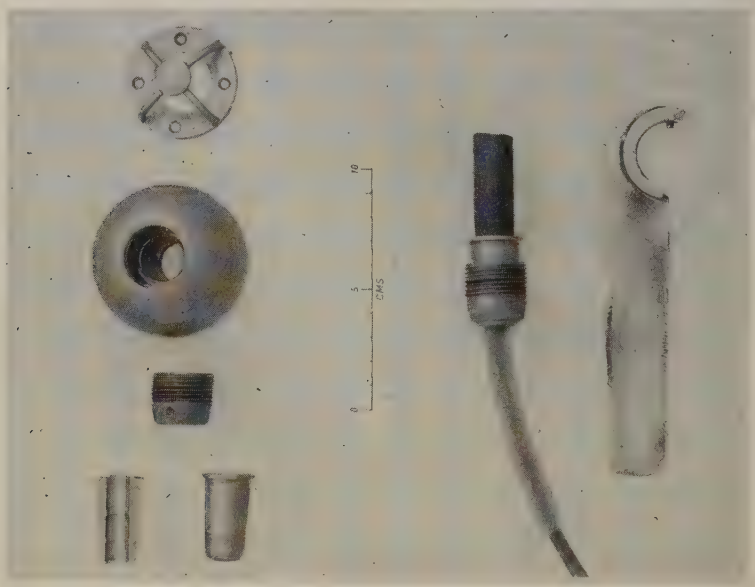

Fig. 4.—Details of end-weight *Left (top to bottom)*: end cage; body of weight; locking block; conical cable wedges. *Middle*: Transducer with wedges and lock in place around cable before screwing into end-weight. *Bottom*: Key for tightening locking-block.

Fig. 5.—Details of cable-weight. *Left:* Components. *Right:* Cable in place in cable-chamber, with rubber locking-block in the centre.

It is particularly important that, when the two weights are in place in the animal, the cable between them should not drag on the reticulo-ruminal fold. A sufficient length is allowed for the fold to be cleared by about 5 cm in an adult cow when both reticulum and anterior rumen sac are relaxed.

As an alternative to the standard end-weight, a second cable-weight may be used, the transducer being taped* back on its own cable, facing upwards. However, the records will differ from those obtained using the standard end-weight.

Anterior Rumen Sac

The standard recording point used in the anterior rumen sac is the floor, about 6 cm back from its lowest point (position 2, Fig. 8). The transducer is taped to the cable of the reticulum transducer, just before the lead enters the cable-weight (see Fig. 6).

If no record is being made from the reticulum, either a normal end-weight, or the alternative described above, may be used.

Dorsal Rumen Sac

The standard recording point in the dorsal rumen sac is alongside the fistula (position 3, Fig. 8). The transducer is simply held in posi-

* Lassometic self-adhesive tape (manufactured by Herts. Pharmaceutical Co. Ltd., Welwyn Garden City, Herts., England) is satisfactory for taping transducers and cables.

Fig. 6.—Complete unit for recording from the reticulum (transducer in end-weight) and the anterior rumen sac (transducer taped behind cable-weight).

Fig. 7.—Method of using a cable-weight in place of the end-weight.

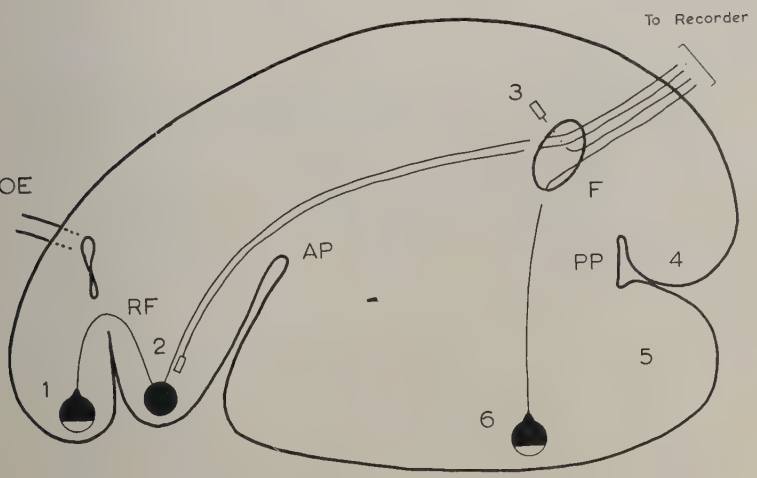

Fig. 8.—Recording points in the reticulo-rumen: 1—reticulum; 2—anterior rumen sac; 3—dorsal rumen sac; 4—posterior dorsal blind sac of rumen; 5—posterior ventral blind sac of rumen; 6—ventral rumen sac. AP—anterior pillar; F—fistula; OE—oesophagus; PP—posterior pillar; RF—reticulo ruminal fold.

tion by the sealing bung (see below). To give extra end-on protection, an outer sleeve about 2 cm longer than the transducer should be used.

Posterior Dorsal Blind Sac

The standard recording point in the posterior dorsal blind sac is the lowest point in this diverticulum (position 4, Fig. 8). A normal end-weight may be used to keep the transducer in position.

Ventral Rumen Sac

The standard recording point in the ventral rumen sac is the floor of the rumen vertically below the fistula (position 6, Fig. 8). A normal end-weight is used to keep the transducer in place.

Roving Transducer

During internal palpation of rumen activity, a roving transducer is useful. For this purpose, a transducer is mounted in a stainless steel tube 16 mm in diameter and 160 mm long which serves both for protection and as a handle. The lower end of this tube is closed by a soft rubber cap and behind this, 4 holes, 5 mm in diameter, give access to the transducer which lies immediately within. The transducer is kept in position by a split-rubber tubing spacer over the cable in the body of the tube, and by a split rubber bung firmly inserted in the mouth of the tube.

Modifications to the Cannula

Since recording is usually carried out with the fistula closed, a convenient method of anchoring the cables at the cannula is required

which does not interfere with sealing. The Balch and Johnson plug has therefore been modified as follows (see Fig. 9, 10): A hole about 8 cm diameter is cut in the centre of the top (outer) plate and one of 5.75 cm in the lower (inner plate), the latter being cut with a large cork-borer. In the lower hole is clamped a brass socket tapered to match a large rubber bung through which pass the cables. The bung has five holes drilled through it, four a neat fit for the cables and one of 2 cm diameter. Leading into each small hole from the side of the bung for its full length is a slit. By simply squeezing the bung and collapsing the large hole, these slits may be opened one at a time, allowing the cables to be quickly slipped in place. The large hole is closed by an expandable stopper which also serves to tighten and seal cables and bung in place.

With the Balch and Johnson cannula, there is normally a small amount of leakage between the body wall of the animal and the cannula. For most purposes, this is of no consequence. However, if necessary, it may be eliminated by packing under the outer flange of the cannula a long roll of cotton wool previously soaked in water.

Results and Discussion

A portion of a typical record from a fistulated cow is shown in Fig. 11.

Over the past 3 years, the equipment has been used in a variety of investigations centring on the ruminant stomach; a general account of some of this work has already been published (Reid and Cornwall 1959).

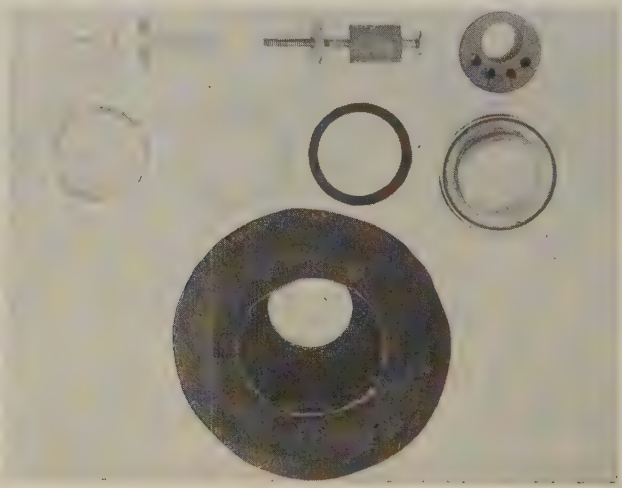

Fig. 9.—Components of modified cannula plug. *Top:* Rubber bung and components of expandable plug. *Middle* (*l to r*): Brass socket; soft rubber washer; polythene washer; threaded brass locking ring. *Below:* Modified Balch and Johnson plug.

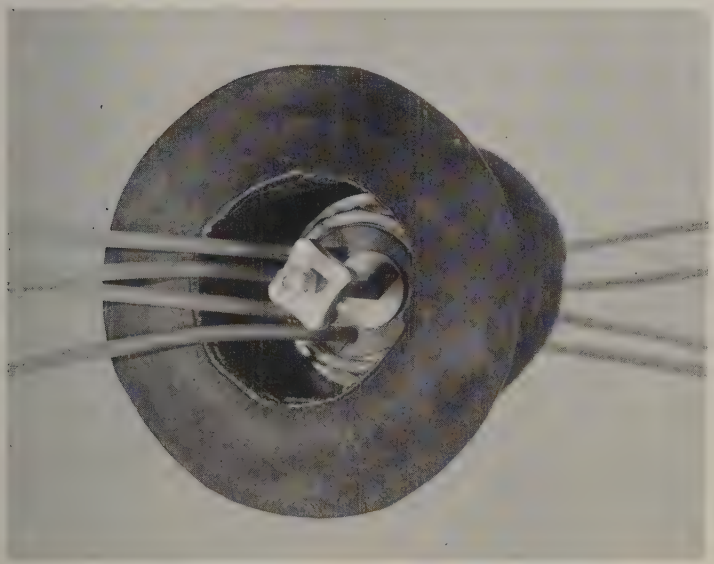

Fig. 10.—Details of construction of modified cannula plug.

As well as intragastric pressures, recordings have been made of arterial blood pressures, perfusion pressures, salivary flow, jaw movements, girth changes, and the mechanical activity of various structures in the reticulo-rumen, using a pressure chamber (see Appendix) and suitable ancillary apparatus. The basic unit is, in fact, widely adaptable; within limits, any physiological variable capable of being expressed as a change in pressure, resistance, or displacement may be recorded. In addition, phenomena which can be made to operate contacts may be registered by the marker pens, if necessary with the aid of a relay.

Performance of the Electrical Equipment

The electrical equipment has proved adequate for all the purposes to which it has so far been put. In operation, it has been convenient to use and reliable. The combination of a low volume/pressure coefficient and high stability and sensitivity has not only been of value in recording pressure fluctuations in the reticulo-rumen more accurately, it has also made possible the development of measuring techniques based on hydrostatic pressure changes, e.g. the registration of vertical displacement by means of a flexible column of liquid (Reid and Cornwall 1959) and of salivary flow with an automatic siphon.

In stability trials with the transducers lying undisturbed in a constant temperature bath, the drift in the zero line has been equivalent to ± 2 mm Hg in 36 h. The change in gain over the same period has been only ± 3%. When recording from the reticulo-rumen, however, where the transducer is subject to continual buffeting, rather more drift of the base line may occur, occasionally as much as ± 10 mm Hg.

Stability appears to be a transducer characteristic; certain transducers can be relied upon to remain very stable, their associated amplifiers requiring almost no readjustment before use on successive days for periods of a week. The major source of drift is the latex sheath which tends to swell and become distorted after it has been in the rumen for

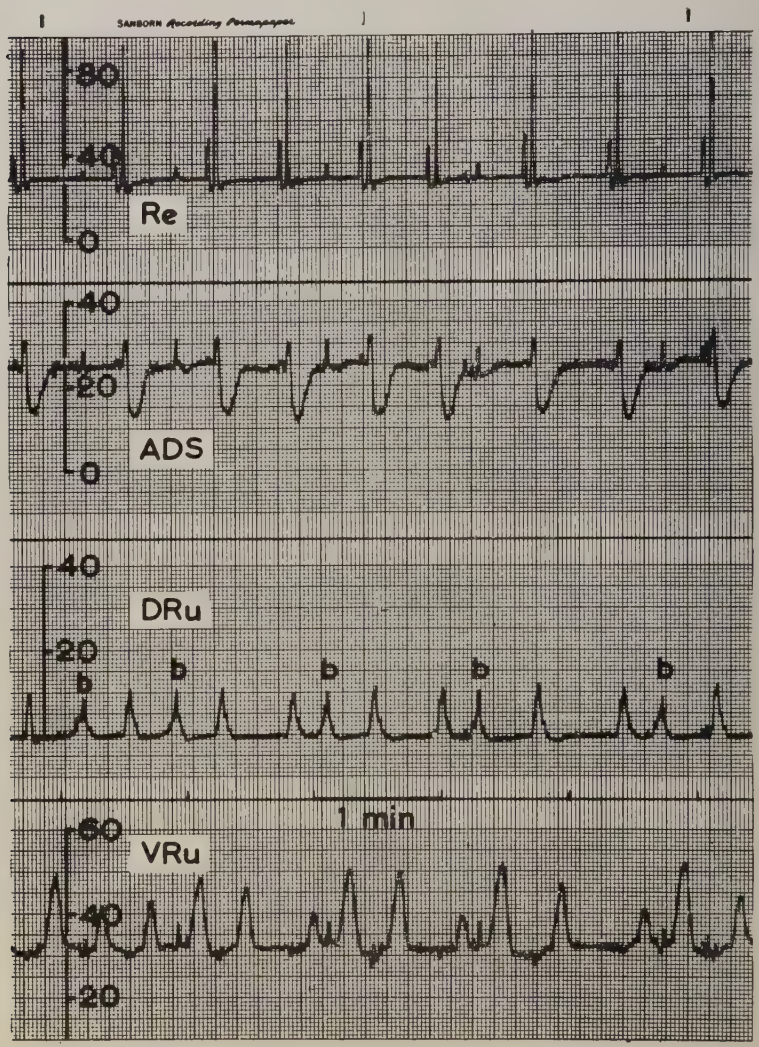

Fig. 11.—Typical record showing pressures registered simultaneously in reticulum (Re), anterior rumen sac (ADS), dorsal rumen sac (DRu), and the ventral rumen sac (VRu). Eructations are marked b.

a time. Much thought has been given to alternative methods of covering and sealing but no perfect solution has yet been found.

Some 300 recordings have now been made with the equipment. The mechanical stresses involved have been satisfactorily withstood by the transducers. A minor weakness in the present design is that the connecting wires must take a considerable proportion of any tension on the cables. If the latter become entangled in masses of ingesta, the tension may be quite large and occasionally the leads have pulled off the connection tags on the transducer. While it would be an advantage to have a special tension wire running through the cable, no satisfactory method has been found for attaching it to the transducer casing.

The Effects of Recording on the Animal

The animals rapidly become accustomed to the handling involved in placing the transducers in position, although at first, they may eat a little less than usual.

Any method involving the location of recording apparatus in the stomach introduces the possibility of modifying the activity of the reticulo-rumen. Stretch in particular is a potent stimulus in the gastro-intestinal tract. Responses of the ruminant stomach to both stretch and tactile stimuli have been described and include changes in the force and frequency of contractions of different compartments, the occurrence of regurgitation and rumination, and changes in the rate of salivation (see Schalk and Amadon 1928); Clark and Weiss 1952; Ash and Kay 1957; Comline and Titchen 1957; Titchen 1958; Stevens and Sellers 1959). Both excitation and inhibition may occur; the exact pattern of effects depends on the nature and strength of the stimulus, its location, and its duration. The cardia, the oesophageal groove, and the reticulo-ruminal fold are the most sensitive structures (Ash and Kay 1957).

Insertion of the anchor weights into the anterior reticulo-rumen of cows starved overnight is often followed by a period of rumination lasting several minutes. Rumination at other times during the day appears to be normally related to the presence of feed in the stomach. Comparisons of records made with and without the weights in place have shown no gross differences in the rate of contraction of the rumen. Nevertheless, the possibility of more subtle effects remains. Reliable evidence of such changes is difficult to obtain, since the mere handling necessary to carry out any experiment may itself temporarily modify the activity of the stomach.

While the use of weights is not an ideal solution to the problem of anchoring objects in the stomach, practical and theoretical objections can also be raised to the possible alternatives—introduction of the transducers through the oesophagus, the use of semi-rigid arms attached to the cannula, or multiple fistulation.

The size of the weights necessary was determined experimentally. Smaller weights were displaced during feeding; even those finally used may be pulled out of place if the feed is very fibrous. Weights of similar size have been used by Balch *et al.* (1951) and Balch 1952.

The Interpretation of Pressure Records from the Reticulo-rumen

The major source of the pressure fluctuations in the reticulo-rumen are the contractions of the various compartments. The pressures developed depend primarily on two variables, the strength of the contractions themselves and the resistance offered by the stomach contents, and secondarily on the hydrostatic effects of changes in level of the compartments and transfer of their contents. Extra-gastric events such as contractions of the diaphragm or body wall, e.g. during respiration, are usually only a minor cause of pressure change; but whereas the effects of the stomach contractions are largely restricted to the compartment in which they arise, extra-gastric events tend to affect pressures in the entire viscus simultaneously.

The records obtained depend on the exact location of the recording points within the compartments and the technique used. Thus, records made from the floor of the reticulum or of the anterior sac of the rumen show the effects of the large changes of level occurring during contraction, which do not appear in records from a fixed point. Again, some pressure fluctuations in the posterior dorsal blind sac are so restricted that they can only be recorded from the deepest point in the compartment. The location of the standard recording points used here was determined by the need to be able to place the transducers at the same definite points on successive occasions, and the ability of the anchoring system to keep them there. Clearly, if the recording point changes, the value of the records obtained is diminished.

The recording technique itself may introduce artefacts; the large spikes in the reticulum record shown in Fig. 11 are in part the result of momentum pressing the end-weight into the floor of the compartment as the second contraction commences.

These different factors necessarily affect the interpretation of the records. Because several of the variables are unknown, quantitative analysis in terms of mechanical activity of the stomach is limited. Even as a general index of this activity, the records are deficient in that contraction of some structures such as the pillars and the posterior ventral blind sac may create little if any detectable pressure change (Schalk and Amadon 1928; Phillipson and Reid, unpublished results). Some of these disadvantages may be avoided by the use of more direct methods, such as the recording of vertical displacement (Reid and Cornwall 1959 and in preparation) or of the contractions of partial exteriorisations of the stomach (Reid and Titchen in press). The latter technique has the added advantage of being less likely to modify the activity being recorded.

Acknowledgments

The construction and development of this apparatus would not have been possible without the generous donation towards its cost made by the New Zealand Dairy Board.

The authors' thanks are due to Mr J. Boyes of the Auckland Industrial Development Laboratories who constructed the transducers, and also the pressure chamber described in the Appendix; to Mr T.

O'Donnell, Plant Chemistry Division, who constructed the weights and the adaptor for the cannula bung, to Messrs R. Stevens (Auckland Industrial Development Laboratory) and H. Drake (Grasslands Division) for the photographs, and to Mr P. Vlieg (Plant Chemistry Division) for the preparation of the figures and for general technical assistance.

The amplifiers were constructed by Electronic Development and Applications Co., Wellington.

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APPENDIX

A Pressure Chamber for Use with the Transducer

A suitable pressure chamber for use with the transducer described in the paper is shown in Figs. 12 and 13. The faces of the perspex ring and the two brass plates are machined flat so that when clamped together, they form a watertight seal. The upper plate has two standard Luer taper sockets. Sealing of the transducer into the lower plate

Fig. 12.—Pressure-chamber for use with transducers described in foregoing paper.

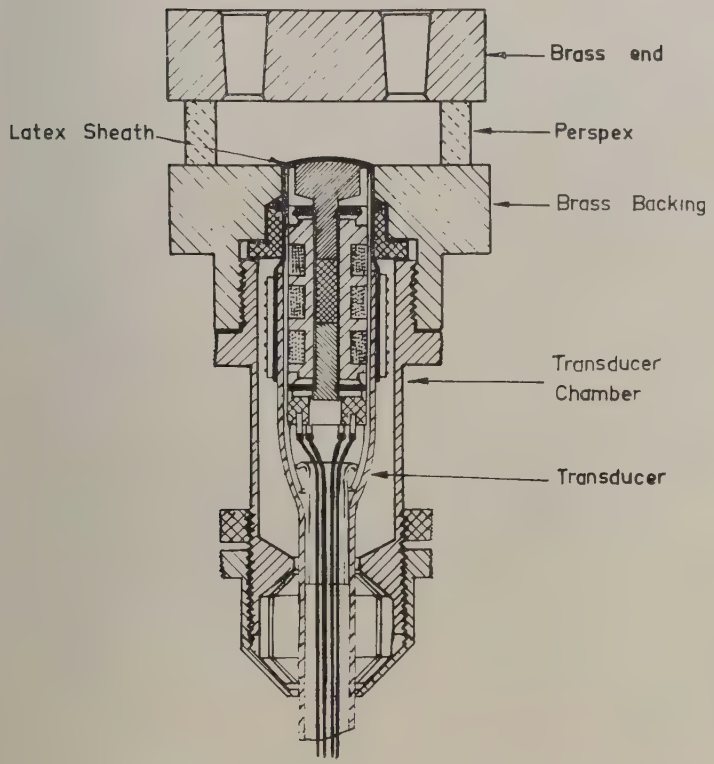

Fig. 13.—Details of construction of pressure-chamber.

is by compression of the "O" ring. A skirt and cable lock protect the transducer from damage during handling.

If desired, the lower plate and the sealing screw may be made of a suitable non-metallic material. This eliminates eddy currents which may in certain circumstances limit the maximum output obtained from the transducer.

The frequency response of the transducer in the chamber when the latter is filled with liquid, is shown in Fig. 14. The response curve of the chamber connected directly to a hydraulic oscillator, shows a gradual fall as the frequency increases (curve A), the response still being 85% at 25 c/s. Normally, however, a conducting system must be introduced between the pressure source and the chamber. A 100 cm length of 2 mm bore, stiff polyvinyl chloride tubing is suitable for this purpose, but the system then has a resonance at about 15 c/s (curve B). This may be damped out by including a 22 gauge syringe needle with a bore diameter of 0.015 in., 26 mm long, in the conducting line (curve C). The resulting frequency response will allow adequate registration of the ventricular pulse wave.

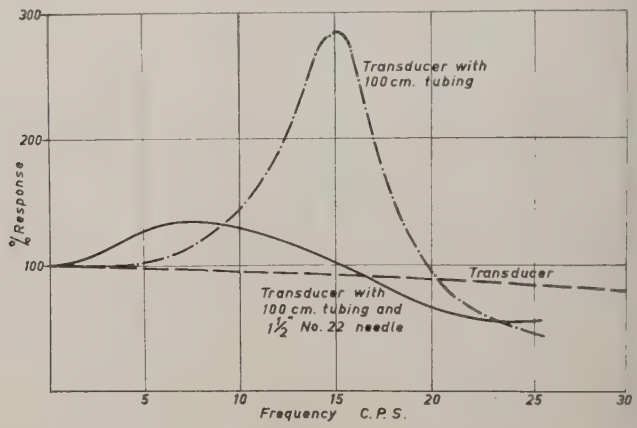

Fig. 14.—Frequency response of assembled transducer and chamber; A (solid line)—chamber connected directly to pressure source; B (dot, dash)—chamber connected by 100 cm of 2 mm bore, stiff polyvinyl chloride tubing to pressure source; C (broken line)—as B, but hydraulically damped by the introduction of a 22 gauge syringe needle 26 mm long.

BLOAT IN CATTLE

XVIII. THE PROTEINS OF BOVINE SALIVA

By J. W. Lyttleton, Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 2 November 1959*)

Summary

The protein composition of a number of samples of bovine saliva has been studied by electrophoretic analysis. The major component in most samples is a mucoprotein containing sialic acid.

This mucoprotein has been isolated by fractionation of saliva, and some of its physical properties have been studied.

Introduction

Previous papers of this series (Johns 1954; Reid and Johns 1957) ve proposed that bloat in cattle is caused by a foam of abnormally ..h mechanical strength being formed within the rumen during rmentation of the ingesta.

It is known that animals vary in their susceptibility to the ail- nt when feeding on the same pasture, and the susceptibility appears e at least in part an inherited characteristic. It has been suggested ns 1954) that part of the contribution of the animal to the foam duction could be due to the animal's saliva. It has been shown in oratory experiments (Mangan 1959) that salivary proteins are power- foaming agents, so may contribute to the stability of the foam in ie rumen.

The work described in this paper was undertaken to learn more ut the different protein fractions which occur in bovine saliva, and see whether recognised differences between animals in susceptibility bloat were paralleled by a similar difference in the protein composi- on of the saliva.

Materials and Methods

Nitrogen was determined by the micro-Kjeldahl method.

Phosphorus was determined by the micro-modification of the method Allen (1940).

Total sialic acid was estimated by the resorcinol method (Svenholm 1957). Free sialic acid was determined by the pyrrole test Aminoff 1959).

Electrophoretic analyses were carried out in a moving-boundary paratus of the Tiselius type, constructed by LKB-Produkter Ltd. sphate buffer, pH 7.5, ionic strength 0.1, was routinely used for liva analyses.

Ultracentrifugal analyses were carried out in an air-driven ultra- trifuge of the Bauer-Pickels (1937) type, at 60,000 rev/min.

Saliva was collected from cows while they were feeding in the stalls used for experimental work with bloat (Johns 1954). It was withdrawn from the lower part of the mouth by suction applied to a perforated metal tube, shaped to fit under the tongue, in which position it did not interfere with the normal movements while feeding. There was no apparent contamination of the saliva samples with plant material even when lush red clover was being eaten (Mr C. S. W. Reid, pers. comm.).

When electrophoretic analysis alone was to be carried out, 50–70 ml saliva were collected; but where a larger sample was required for fractionation, up to 300 ml could be taken from a cow during a single feed.

Concentration of Salivary Protein

Saliva as collected normally contained between 1.0 and 2.0 mg protein per ml. This is undesirably dilute for operation of the moving-boundary electrophoresis apparatus, so it was necessary to concentrate the salivary protein prior to analysis. This was normally done by precipitating the total protein from the saliva by addition of Na_2SO_4 to 32% (w/v) at pH 5.5 (Mangan 1959), but identical electrophoretic patterns were obtained if the concentration was carried out by freeze-drying, or by dialysis under reduced pressure.

Results

Electrophoretic analysis of bovine saliva regularly showed two main components, A and B (Fig. 1), which were well defined in the ascending limb, and less well resolved in the descending limb. Following the main components, most saliva samples contained a certain amount of less rapidly moving material, rather poorly resolved (component C).

In Table 1 is given the percentage composition of a number of saliva samples, taken from different cows on different occasions. The protein concentration of the saliva samples, prior to concentration, is also given.

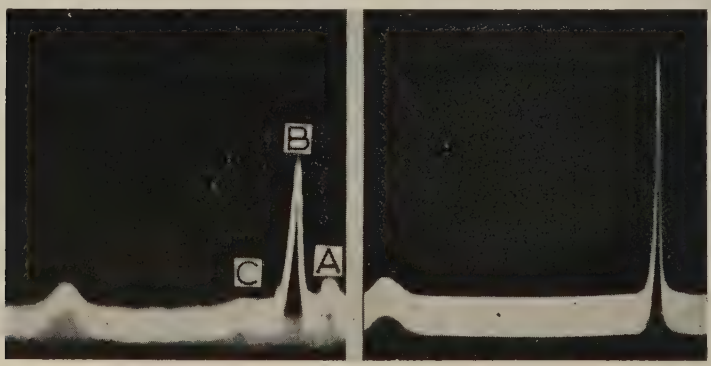

Fig. 1.—Electrophoretic diagrams. (a) Bovine saliva. (b) Salivary mucoprotein. Diagrams are from ascending limb of U-tube. Migration from left to right.

TABLE 1. ELECTROPHORETIC ANALYSES OF SALIVA SAMPLES

Date	Cow	Protein Level in Saliva (mg/ml)	Percentage of Components Present		
			A	B	C
9/ 8/57	48	—	34	45	21
19/ 8/57	294	—	19	65	16
23/ 8/57	293	1.6	17	66	17
26/ 8/57	294	1.2	16	63	21
27/ 8/57	293	1.7	15	58	27
28/ 8/57	293	1.6	20	63	17
29/ 8/57	293	1.7	20	58	22
23/ 9/57	90	2.1	26	61	13
24/ 9/57	90	2.2	23	62	15
25/ 9/57	48	1.3	43	41	16
26/ 9/57	48	1.1	41	43	16
3/10/57	48	1.8	47	43	10
22/10/57	1	0.9	29	62	9
27/11/57	47	—	48	41	11
21/ 5/58	47	—	30	54	16
22/ 5/58	90	—	23	60	17

Nature of Protein Components

Curtain and Pye (1955) have isolated from bovine submaxillary glands a mucoprotein which contained sialic acid as part of its carbohydrate moiety. As the major part of the protein in mixed bovine saliva originates in secretion from the submaxillary gland (Phillipson and Mangan 1959), it was of interest to discover if either of the two major components of saliva contained sialic acid, as would be expected if it was related to the mucoprotein extracted directly from the gland. Accordingly a sample of saliva from Cow 48, containing nearly equal amounts of components A and B and less than 5% of C was subjected to electrophoresis, and pure samples of components A and B were isolated from the cell at the end of the run. Analysis of these fractions indicated that whereas B contained sialic acid, component A contained none. As the presence of sialic acid is typical of mucoproteins, component B will henceforth be referred to as salivary mucoprotein.

Purification of Salivary Mucoprotein

Fresh saliva was acidified to pH 4.5 with acetic acid, then centrifuged at 10,000 rev/min in the No. 20 rotor of the Spinco Model L ultracentrifuge to remove the precipitate. This rather severe centrifugation was necessary because of the high viscosity of the saliva. The supernatant was then cooled to 4°c, and 2.5 volumes of cold 95% (v/v) ethanol added slowly with constant stirring. A floccular precipitate developed, which was allowed to settle, and the supernatant poured off. This precipitate was stirred with a little distilled water until a uniform viscous solution was obtained. Finally, this was clarified in the No. 40 Spinco rotor at 25,000 rev/min for 10 min, and the clear supernatant freeze-dried.

Properties of Salivary Mucoprotein

Freeze-dried mucoprotein, prepared as above, dissolved readily in water, giving a viscous solution. Electrophoretic analysis, over a pH range of 7.5 to 2.0, revealed a single peak (Fig. 1), with a mobility at pH 7.5 corresponding to that of component B of the unfractionated saliva. The variation of mobility with pH is shown in Fig. 2. In the ultracentrifuge, salivary mucoprotein sedimented as a single component. In 0.2 M NaCl, at pH 7.5, the sedimentation coefficient extrapolates to 3.7 S at infinite dilution.

An analysis of this purified material in terms of its amino-acid and carbohydrate constituents is in progress and will be published later (Mangan and Bailey, in preparation).

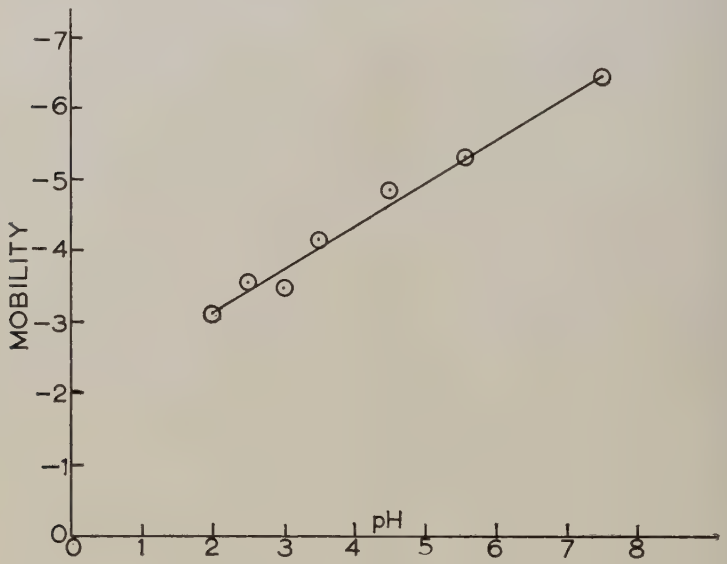

Fig. 2—Electrophoretic mobility of bovine salivary mucoprotein at different pH values. Buffers used (all at ionic strength 0.1).
pH 7.5 KH_2PO_4/Na_2HPO_4
pH 3.5–5.6 Na acetate/acetic acid
pH 2 –3 glycine/HCl
Mobilities expressed in units of $10^{-5}cm^2/volt\ sec.$

The mucoprotein contains no detectable sulphur.* When prepared as above it contains about 0.5 μg phosphorus/mg mucoprotein. This appears to be due to contamination with phosphate from saliva, as the phosphorus can be completely removed by passing through a column of anion exchange resin IRA400 in the chloride form. This treatment causes no loss of protein nitrogen, and does not modify the electrophoretic mobility of the mucoprotein.

* This analysis was carried out by Dr A. Campbell, Chemistry Department, Otago University.

As with the submaxillary gland mucoprotein, sialic acid may be liberated by heating at 100°c for 30 min at pH2 (Svennerholm 1958) or by incubating with the sialic-acid-splitting enzyme derived from *Clostridium perfringens* (Popenoe and Drew 1957).

Discussion

The data in Table 1 indicate that in most cases the protein composition of the saliva samples taken during feeding was relatively constant. In the cases of the twin cows 47 and 48, however, a lower proportion of the salivary mucoprotein (component B) was present, and the front component (A) was increased in amount. It is interesting to note that of the cows that have been used for experiments on bloat at this Division, cows 47 and 48 are on occasions noticeably less prone to bloat than the other animals.

Results to date show a similarity between members of twin sets and differences between twin sets in the salivary-protein pattern. This pattern seems thus to be inherited, as is the tendency to bloat. It would be desirable to attempt to establish a correlation between the levels of salivary mucoprotein and this bloating tendency. To obtain sufficient saliva for electrophoretic analysis, however, requires that the animal be well accustomed to handling during stall feeding, and extension to a large number of cows will be difficult.

It is very probable that this salivary mucoprotein is closely related to, if not identical with, the mucoprotein extracted directly from the submaxillary glands (Curtain and Pye 1955). Confirmation of this must await detailed chemical analysis. The steps used in isolating the mucoprotein from saliva are milder than those needed from the glandular extract, and lead to an electrophoretically more homogeneous product.

The electrochemical properties of salivary mucoprotein are somewhat unusual. At pH 2.0 it still has a high negative electrophoretic mobility, indicating the presence of negatively charged groups on the molecule. As neither sulphate nor phosphate groups are present, this charge is presumably due to the sialic acid, which in the uncombined form has a pK of 2.7 (Svennerholm and Svennerholm 1958).

The sialic acid content of the salivary mucoprotein is not known, but if it is as high as the 22% found for submaxillary gland mucoprotein (Gottschalk and Graham 1958), the highly viscous nature of the protein solutions might be explained in terms of strong molecular interactions brought about by the existence of so many negatively charged groups on each protein molecule.

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THE DISTRIBUTION OF MOLYBDENUM IN THE SOILS OF NEW ZEALAND

I. SOILS OF THE NORTH ISLAND

By J. L. Grigg, Winchmore Irrigation Research Station*

(*Received for publication, 12 August 1959*)

Summary

Soils of the North Island have been examined for their molybdenum content. Wide variations in levels of molybdenum extracted by acid ammonium oxalate have been found for individual soil sets, but some important differences are noticeable between genetic soil groups.

Total molybdenum levels in certain North Auckland soil suites are shown to decrease as the soils become more leached.

In a comparison of sedimentary rocks, mudstones give soils higher in acid-oxalate-soluble molybdenum than do sandstones or greywackes. In the volcanic and igneous groups, yellow-brown loams from andesitic and rhyolitic ash are generally much higher than yellow-brown pumice soils from rhyolitic ash. Immature basalt soils have high levels of available molybdenum.

Some correlations have been shown with the type of clay minerals in the soils.

Response of pasture to molybdate topdressing has been discussed in relation to the different soil groups.

A comparison of soil test levels and pasture responses has shown that acid ammonium oxalate is not reliable for diagnosing deficiencies in that it extracts a portion of the iron-bound molybdate which would not be available to plants.

Introduction

Following early indications (Davies *et al.* 1951; Lobb 1953) that remarkable improvements in pastures and crops would be obtained by the application of small quantities of molybdenum, a concentrated programme of research has been undertaken by officers of the Extension Division, Department of Agriculture, to study its place in the farming economy of New Zealand. As part of this programme, a survey was made of the molybdenum content of New Zealand soils to define those areas where molybdenum would be a useful auxiliary fertiliser.

Early studies showed that levels of total soil molybdenum did not correlate well with field responses, and a method of extracting a readily soluble fraction of the soil molybdenum was devised (Grigg 1953a, b, c). This method is based on the replacement of molybdate held on the clay complex as an exchangeable anion by oxalate from an acid ammonium oxalate solution of pH 3.3.

A large number of analyses for acid-oxalate-soluble molybdenum has accumulated, and it is the purpose of this paper to classify these and if possible to draw some general conclusions from the levels found.

* This work was carried out while the author was at the Rukuhia Soil Research Station, Hamilton.

Total molybdenum levels have been determined by the method of Perrin (1947) for a few soils in North Auckland and these are also discussed briefly.

Origin of Samples

Soil samples have been collected from three sources:

(1) From molybdenum topdressing trials, mainly on pasture, as they were laid down. Soil types have been checked by officers of the New Zealand Soil Bureau.

(2) From farms in connection with the Extension Division Advisory Soil Testing Service. These samples were collected by field officers of the Division who have a sound knowledge of the soils of their own district.

(3) From the Superintendent, Wallaceville Animal Research Station. Samples were collected from defined soil types in company with Soil Bureau officers in carrying out a survey of the copper and molybdenum content of pastures (Cunningham *et al.* 1956).

Most samples of soil under pasture have been taken to a 3 in. depth. Some samples from arable ground have been 0–6 in., and a few Soil Bureau samples have been from the A horizon of an undisturbed profile.

Method of Presentation of Results

A classification of the soils with emphasis on parent material has been selected as the most suitable for the purpose of this paper. Such a scheme is in use by the Department of Agriculture for coding results of advisory soil tests on Powers Samas punch cards, and is used here with modifications. An attempt has been made to separate, where possible, the effects of weathering and leaching.

Basic soil data have been taken from Soil Bureau Bulletin 5, "General Survey of the Soils of North Island, New Zealand", from the Genetic Soil Map of New Zealand (Taylor 1948), and from other more detailed local soil surveys.

Analytical results are considered in suites of soils derived from the same or similar parent material, where possible, in the divisions given below. Taylor's (1948) genetic group numbers are shown in parentheses. Skeletal soils are included with their nearest zonal counterpart.

(1) Soils derived from sedimentary and acid igneous rocks:

Rendzinas	(11)
Yellow-grey earths	(2b, 2f, 2h)
Yellow-brown earths and podzols	(5a–5c, 6a–6c, 7a, 7b)
Yellow-brown sands	(12)

(2) Soils derived from volcanic ash and basic igneous rocks.

This includes the following groups:

Recent soils from volcanic ash	(20b)
Yellow-brown pumice soils from rhyolitic ash	(13a, 13b)
Yellow-brown loams from rhyolitic and andesitic ash	(14a–14d)
Brown granular loams and clays from volcanic ash and from andesite	(15a, 15b)
Red-brown loams from basalt	(16a–16c)

(3) Soils of the plains:

Recent soils from alluvium	(20a)
Gley soils	(17)
Saline gley soils	(18)
Organic soils	(19)

In order to give a detailed picture of the range and distribution of molybdenum levels for the subdivisions concerned, the mean (M) and its standard deviation (S) have been listed. The interval $M \pm S$ includes about $\frac{2}{3}$ of the samples in the group.

The number of samples within various ranges of molybdenum content are also given to show the distribution of values.

Total Molybdenum Levels

On certain parent rocks in the North Auckland district it has been possible to map suites of soils of varying degree of development, reflecting the formation of the soils under an original environment of mull-forming, mor-forming, or mixed forest. The results in Table 1 show that there is a progressive loss of molybdenum as leaching and podzolisation proceed. This is very marked for the Omu, Marua, and Te Kie suites, in the levels of both total and acid-oxalate-soluble molybdenum, but is not so marked in the Puhoi suite where the sandstone parent material is low in molybdenum at all stages of development.

The few examples from the Kiripaka suite of soils formed on basalt illustrate a different mechanism of soil formation. As pointed out by Taylor *et al.* (1956), weathering is the dominant factor on this parent material, leading to accumulation of sesquioxides rather than podzolisation. The total molybdenum levels found are in agreement with figures given by Wells (1956) who showed that the youngest members of the sequence had very high molybdenum levels, the semimature members had lower amounts, while the mature members with ironstone nodules again contained higher amounts, reflecting the tendency of molybdenum to accumulate with sesquioxides.

Available Molybdenum Levels as Measured by Oxalate Solubility

Soils Derived from Sedimentary Rocks and Acid Igneous Rocks

Table 2 lists these soils for the North Auckland district and Table 3 for the rest of the North Island. This separation is made because the North Auckland soils can be classified into suites of soils, each on one type of parent material, more completely than can the soils for the rest of the Island.

North Auckland Soils. Soils from parent materials containing limestone in North Auckland are predominantly below 0.10 p.p.m., although a few high values raise the mean to 0.15 p.p.m.

On coastal sand dunes recently fixed by vegetation (Pinaki suite), very low molybdenum levels are found. As the dunes consolidate and weather (immature and semimature stages), higher levels are found, especially on the sandy loams and sandy clay loams, but, as podzolisation occurs, exceedingly low levels are again reached.

In the Omu suite (claystone) there is a progressive reduction in molybdenum levels with maturing of the soil as was mentioned earlier. Although only 3 samples represent the young and immature stages, the trend is quite marked.

Puhoi and Waiotira suites from sandstone show that even the least leached members (skeletal to immature stages) have low levels of molybdenum. There is nevertheless a decrease in the submature and mature stages of the Waiotira suite.

Soils of the Marua suite, which are derived from greywacke, are also low to very low in molybdenum. Here again the two samples of the submature member (Hukerenui silt loam, yellow subsoil phase, Hr) are exceedingly low.

Other North Island Soils. Results for the rest of the North Island in Table 3 show many interesting features.

Yellow-grey earths from alluvium, muddy sandstone, etc., are generally high in molybdenum, reflecting the higher initial molybdenum content and the lower degree of leaching of these soils. Yellow-grey earths in the South Island*, in contrast, are very low in molybdenum. The group classed as yellow-grey earths transitional to yellow-brown earths gives much lower levels than North Island yellow-grey earths and slight responses have been obtained on some of these types.

The yellow-brown earth to podzol sequences are subdivided according to whether the parent material is predominantly limestone, sand, mudstone, sandstone, or greywacke. Complexes of these with volcanic ash are considered later in the table.

The coastal dune sand soils are similar to those in North Auckland. Where there is admixture of volcanic ash or pumice much higher levels of molybdenum are found.

In the mudstone group, the mean for skeletal soils is very high. Young and immature soils show lower values, but the three groups overlap.

As in North Auckland, soils derived from sandstone are lower than those from mudstone, but the skeletal group here is higher than in the Puhoi and Waiotira suites.

Soils formed on greywacke tend to be slightly higher than soils of the Marua suite in North Auckland and there is no noticeable diminution of level with greater degree of leaching.

A subdivision of those soils where volcanic ash is present shows a similar situation, molybdenum levels in mudstones being little affected by the ash, whereas in sandstones and greywackes the mean levels are higher.

The Takapau suite of soils from sandstone and greywacke alluvium with pumiceous ash gives mean values very similar to the complexes of ash with sedimentary rocks.

* Molybdenum status of South Island soils will be dealt with in Part II of this series.

Soils Derived from Volcanic Ash and from Intermediate and Basic Volcanic Rocks

As will be seen from Table 4 the mean content of oxalate-soluble molybdenum in these soil groups is in general much higher than in the sedimentary rocks.

The pumice soils of the central plateau region are in general well supplied with molybdenum. Some members of the Taupo and Kaharoa suites give low figures. The reason for this may lie in the fact that these soils are lighter in texture.

An area of an early phase of the Taupo ash shower north of Wairoa has been entered separately because of much higher molybdenum levels found both in the soil and the pasture (Cunningham 1955).

Yellow-brown loams formed from the Waihi and Tirau showers of rhyolitic ash have higher levels of available molybdenum than any other soil group (apart from a few samples examined in the Kiripaka suite). Levels as high as 1.5 p.p.m. have been obtained on Katikati sandy loam (soil set 54). These results agree with Wells' (1956) finding that yellow-brown loams have a high retentive power for molybdate.

Andesitic showers in the western districts of the North Island from Raglan to Wanganui give rise to soils having molybdenum levels which, while still high, are considerably lower than those of the Waihi and Tirau suites.

The brown granular clays of the Hamilton ash suite present some interesting features. Soils classed as Hamilton clay loam (83) fall into two distinct groups. In the Hamilton area they ranged from 0.54 to 1.42 while in the Franklin County the levels were 0.15 to 0.27 p.p.m. Other soil sets derived from Hamilton ash ranged in molybdenum content from 0.09 to 0.33 p.p.m. with the exception of Patumahoe clay loam (82- Hamilton ash over basalt and sediments) which gave very high figures (0.35–0.65 p.p.m.). Grouping these soils together results in a bimodal frequency distribution.

Additional evidence for differences in Hamilton ash between the two localities mentioned is found in the soil potassium levels (Rukuhia Soil Research Station, unpublished data).

In contrast to the high levels found in the yellow-brown loams and brown granular loams and clays derived from andesitic ash, the brown granular loams and clays of North Auckland, derived from andesitic breccias and andesitic basalt, etc., are considerably lower in molybdenum. This difference may be connected with the kaolinitic nature of this latter group of brown granular clays, whereas in yellow-brown loams the predominant clay minerals are amorphous hydrous oxides of iron and aluminium and allophane, (Fieldes and Williamson 1955).

Young and immature soils from basalt (Ohaeawai silt loam and Whatatiri clay loam) are listed separately as they give very high levels of available molybdenum. The semimature and mature members of the suite are very low, even though their total molybdenum content may be

very high (cf. Table 1). These figures again confirm Wells' (1956) finding that weakly leached basalt soils have a high retentive power for molybdate while strongly leached members of the suite have a lower retention, in agreement with the changing of the amorphous hydrous oxides of aluminium, iron, and titanium into fine crystals of gibbsite, goethite, boemite, and anatase (Fieldes and Williamson 1955).

Alluvial, Saline, and Organic Soils

Although there are some low values, the majority of soils in this group are also well provided with molybdenum, as will be seen from Table 5. Gley soils derived from volcanic-ash alluvium are much higher in oxalate-soluble-molybdenum than those derived from alluvium from sedimentary rocks. It is not possible to divide the recent alluvial soils into those from sediments and those from ash with any degree of certainty owing to the incomplete differentiation of recent soils in the general survey.

The saline-soil samples are mainly from the Ahuriri lagoon reclamation area. High levels of pasture molybdenum here cause stock trouble.

It is not known whether the acid-oxalate extraction gives a true picture of molybdenum solubility on organic soils. Molybdenum may be present in them as an organic complex, not an exchangeable anion, and hence fail to be removed by the oxalate solution.

Response of Pastures to Molybdate Topdressing

At the right of Tables 2, 3, 4, and 5 an assessment has been given of the degree of response obtained in Extension Division field experiments with molybdenum, applied either as sodium molybdate or as molybdenised superphosphate. Where sodium molybdate was used basal superphosphate treatments have been applied in all cases. These data have been obtained from Extension Division records and from published articles (Clarke *et al.* 1957a, b).

The main soil groups on which responses have been obtained are:

(1) Zonal soils formed from sandstone (Omanaia, Puhoi, Waiotira suites) and greywacke (Marua suite), more especially the skeletal to semimature members of these sequences. Such areas are widespread in North Auckland, Raglan County, and in the Wellington and southern Hawke's Bay districts. The submature and mature members, however, give only slight responses easily masked by liming.

(2) Soils formed from mudstones and claystones. In the Omu suite (North Auckland) responses are not obtained on the young and immature members nor on the mature soil, but slight to good responses are obtained for the intermediate members. In the rest of the North Island responses occur only on the immature soils.

(3) Slight responses have been obtained on immature to mature soils formed on consolidated dune sands.

(4) Responses are fairly general on the brown granular loams and clays derived from andesitic rocks in North Auckland. This is in contrast to the brown granular loams and clays from andesitic ash and may be due to a kaolinitic type of soil clay in these soils.

(5) Mature members of the red-brown loams formed from basalt (Kiripaka suite) in North Auckland, which also have a more crystalline form of soil clay.

No responses have been recorded on the following groups of volcanic ash soils:

Yellow-brown pumice soils
Yellow-brown loams
Brown granular loams and clays from volcanic ash.

Although data are meagre, no responses have been recorded on soils derived from limestone and calcareous mudstones.

Recent and gley soils formed from alluvium from either sedimentary rocks or volcanic ash have given no responses.

RELATION BETWEEN SOIL MOLYBDENUM LEVELS AND GROWTH RESPONSE OF PASTURE

In general, those soils giving molybdenum responses have levels of acid-oxalate-soluble molybdenum below 0.20 p.p.m., although not all soils with lower levels have given responses.

Non-responsive soils generally have higher levels, the bulk of samples being greater than 0.15 p.p.m.

In an earlier paper (Grigg 1953a) a tentative response level of 0.14 p.p.m. was suggested, but as more trial results have been obtained it has become apparent that soil type is a better guide to molybdenum response than the chemical test.

It has been postulated that available molybdenum exists as an exchangeable anion (Grigg 1953; Davies 1956). The acid-ammonium-oxalate extraction was developed on the hypothesis that absorbed molybdate would be replaced and complexed by oxalate ions. However, at the reaction chosen (pH 3.3) this reagent dissolves considerable amounts of iron and aluminium oxides.

Wells (1956) considers that hydrous oxides of iron and aluminium formed in the soil by weathering have a high retentive power for the molybdate ion and that this accounts for the low availability of molybdenum in these soils. Jones (1957) has also shown that hydrous ferric oxide has a great adsorptive power for molybdate.

Williams and Moore (1952) showed that the uptake of molybdenum by oats varied directly with the soil reaction, but inversely with the content of ferric iron in the soils, i.e. hydroxyl ions tend to liberate molybdate while iron tends to render it unavailable.

It would thus appear that the acid-ammonium-oxalate extractant dissolves not only the exchangeable molybdate fraction, but also some of the ferric molybdate which would not be available to the plant.

Ammonium oxalate solutions of the same ionic strength (0.275 M) but different pH levels were examined on some zonal soils and intrazonal soils from volcanic ash. Results showed that the neutral solution extracted 27–97% of the amount dissolved by the acid solution on volcanic-ash soils. On zonal soils, however, which would not be expected to have high contents of hydrous iron oxides, the amount extracted was approximately the same with both solutions.

Discussion

Since most of the samples are classed in sets, defined as "groups of soils of like profiles or assemblages of profiles", (N.Z. Soil Bureau Bull. 5, 1954), the wide range of values obtained in individual sets is not unexpected. More precise classification into soil types was not possible for this work.

The levels obtained support the pattern of conditions for molybdenum excess and deficiency postulated by various workers and reviewed by Davies (1956).

It is considered that the publication of these accumulated figures may assist in focussing attention on areas where more experimental work is required.

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TABLE 1. TOTAL AND ACID OXALATE SOLUBLE MOLYBDENUM LEVELS FOR SOME INDIVIDUAL SAMPLES OF NORTH AUCKLAND SOIL TYPES

Soil Suite and Type	Stage of Development	Total Mo p.p.m.	Acid Oxalate Soluble Mo p.p.m.
Yellow-brown earth to podzol sequences			
Omu Suite (Claystone, shale, etc.)			
Aponga clay	Immature-early semimature	4.0	0.29
Waikare brown clay	Semimature-submature	2.0	0.16
Hukerenui silt loam	Submature	1.1	0.10
Wharekohe silt loam	Mature	0.8	0.04
Puhoi Suite (Banded sandstone)			
Atuanui clay loam	Skeletal	0.8	0.08
Puhoi clay loam	Young-immature	1.3	0.12
Whangaripo clay loam	Immature-early semimature	1.1	0.09
Warkworth clay	Semimature	0.9	0.05
Marua Suite (Greywacke)			
Te Ranga clay loam	Skeletal	2.2	0.18
Marua clay loam	Immature-early semimature	1.2	0.11
Rangiora silty clay loam	Semimature	1.0	0.13
Hukerenui silt loam (yellow subsoil phase)	Submature	1.3	0.07
Pinaki Suite (Dune sands)			
Pinaki sand	Young-immature	2.0	0.04
Red Hill sand	Immature-early semimature	1.5	0.04
Te Kopuru sand	Mature	0.9	0.08
Brown granular loam to podzol sequence			
Te Kie Suite (Doleritic breccias & tuffs)			
Te Kie stony loam	Skeletal	3.1	0.20
Takitu clay loam	Young-immature	2.0	0.12
Waimatenui clay	Semimature	2.0	0.11
Awarua clay	Later semimature	2.0	0.09
Rangiuru clay	Later semimature	1.9	0.07
Tinopai sandy loam	Mature	1.8	0.12
Red-brown loam to ironstone soil sequence			
Kiripaka Suite (Basalt)			
Whatatiri clay loam	Young-immature	4.6	1.11
Matarau friable clay	Semimature	1.3	0.03
Okaihau gravelly friable clay*	Submature-mature	6.7	0.08

* Subsoil sample containing ironstone nodules in which molybdenum is known to concentrate.

TABLE 2. Acid Oxalate Soluble Molybdenum Levels of North Auckland Soils from Sedimentary and Acid Igneous Rocks

Parent Material	Suite	Stage of Development †	Soil Types *	Mean (p.p.m.)	S.D.	No. of samples with Mo Level (p.p.m.) from				Response of pasture to molybdate topdressing
						0–.10	.11–.20	.21–.30	>.30	
Limestone Limestone and mudstone Limestone, mudstone, and sandstone Calcareous mudstone	Arapohue Maungaturoto Konoti Whaka	— — — S	AU, MT MO, MK, D KN RV	0.15	0.09	8	3	3	1	No information
Coastal sand dunes	Pinaki	Y–I I–ES M	PN, WH, MD R, Rs, Rl, Rc TK	0.07 0.13 0.03	0.04 0.05 0.02	8 4 16	1 8 —	— 2 —	— — —	PN—Nil R, Rl—Slight Slight
Claystone, shale, etc.	Omu	Y–ES S S–SU SU M	OM, AP MA, OA YK H WK	0.37 0.15 0.13 0.08 0.06	0.11 0.07 0.08 0.03 0.05	- 2 12 9 7	— 8 10 1 1	1 2 3 — —	2 — 1 — —	AP—doubtful No information Good unless limed heavily Slight unless limed heavily No response on limed soils
Sandstone and mudstone	Omanaia	Y–ES	WN, ON, AE	0.15	0.07	1	3	1	--	Excellent

Banded sandstone	Puhoi	SK–I	AN, OI	0.09	0.02	8	3	—	—	Excellent
		I–ES	WR	0.10	0.02	16	12	—	—	Excellent
		S	WA	0.08	0.04	10	2	—	—	Good unless heavily limed
Massive sandstone	Waiotira	SK	WC	0.08	0.00	2	—	—	—	No information
		I–ES	Y, Yr, Ye	0.10	0.03	10	6	—	—	Good
		S	RP	0.10	0.03	4	2	—	—	Good
		S–SU	YKs	0.12	0.04	4	8	—	—	Good to excellent
		SU	Hs	0.07	0.04	4	2	—	—	Slight to good; but ceases at moderately high pH
		M	WKs	0.03	—	1	—	—	—	Probably responds if unlimed
Greywacke	**	SK	TR	0.14	0.04	2	8	1	—	Excellent
	Marua	I–ES	MR	0.14	0.05	4	10	3	—	Good
		S	RA	0.12	0.04	4	6	—	—	Excellent
		SU	Hr	0.06	0.02	2	—	—	—	Good
Dacite	Maungarei	SK	PE	0.16	—	—	1	—	—	No information
		M	PR	0.11	0.05	1	1	—	—	Fair
Terraces from alluvial sediments	Whareora	I–ES	WO	0.09	—	1	—	—	—	Nil
	Waipu	Gley	YU	0.11	0.04	2	3	—	—	Nil

† Stage of development: SK—Skeletal; Y—Young; I—Immature; ES—Early semimature; S—semimature; LS—Late semimature; SU—submature; M—mature.

* Code letters used in North Auckland Soil Survey. See table 6.

** Includes soils of this suite South of Auckland.

TABLE 3. ACID OXALATE SOLUBLE MOLYBDENUM LEVELS OF SOILS FROM SEDIMENTARY AND ACID IGNEOUS ROCK, NORTH ISLAND EXCLUDING NORTH AUCKLAND

Soil Group and Parent Material	Stage of Development †	Soil Sets *	Mean (p.p.m.)	S.D.	No. of samples with Mo level (p.p.m.) from					Response of pasture to molybdate topdressing
					0–.10	.11–.20	.21–.30	.31–.40	> .40	
Y.G.E.**										
Alluvium, muddy sandstone etc.		8–9b	0.30	0.14	1	1	1	3	1	Nil
Y.G.E. transitional to Y.B.E.										
1. Sandstone and pumiceous sandstone		11, 11b, 11c	0.17	0.09	2	3	2	—	—	11 Probable slight response 11b, 11c Probably no response
2. Alluvium		11d, 12, 13, 13a	0.10	0.05	10	12	—	—		Slight
3. Sandy mudstone and mudstone		13b, c, d	0.12	0.05	21	25	2	—		13b and c. Variable, slight to good.
Y.B.E. and Podzols										
1. Limestone	SK	113, 113b	0.15	0.07	2	1	2	—	—	Nil
2. Dune sand	Y	23, 23b, 23c	0.08	0.05	35	6	2	—	—	Nil
Weathered sand plus volcanic ash	I	24 to 24d	0.23	0.14	3	6	4	3	2	Variable. Good response on 24d
3. Mudstones, argillites etc.	SK	114, a, b, d, 115a, 120, 121d	0.28	0.14	7	29	11	11	11	Nil
	Y	25a, c, 26a	0.25	0.11	3	8	8	16	1	Nil
	I	28, cH, 29, f, 31d	0.19	0.13	7	17	7	3	2	28. 29 respond

4. Sandstones	SK	116, b, 117, b, c, 118a	0.17	0.09	9	14	2	4	—	118a, Possible response
	I	32c	0.10	0.05	5	3	—	—	—	Nil
	S	34a, 34b	0.09	0.05	13	8	2	—	—	34b good, 34a doubtful
5. Greywacke	SK	122, 124, b	0.14	0.07	11	16	4	—	—	All greywacke derived soils respond
	S	35a, bH, 41aH, b	0.16	0.08	4	9	3	1	—	
	LS–SU	44c, 46, d	0.15	0.07	6	8	3	—	—	
Y.B.E. Complexes with volcanic ash										
1. Mudstone and ash	SK	114c, 115b	0.25	0.12	—	6	6	3	1	
	Y	25, b	0.21	0.09	2	8	6	4	—	No responses have been recorded on this group of soils
	I	29a, c, dH	0.19	0.07	4	12	4	2	—	
2. Sandstone and ash	SK	117d, 117h	0.25	0.16	1	3	—	2	1	
	I	32H, 32b	0.13	0.03	1	3	—	—	—	
3. Greywacke and ash	SK	122a, 125, 125a	0.16	0.06	1	6	2	—	—	
	LS–SU	44dH, 46CH	0.17	0.05	—	4	1	—	—	
Y.B.L. from sandstone and greywacke alluvium plus pumiceous ash (Takapau suite)	I	74a–76c	0.19	0.08	8	17	22	4	—	No responses of 74 and 75 series
	S	77–78b	0.19	0.09	7	10	12	3	1	Variable responses on 76 and 77 series

Notes: † See footnote to table 2 for key to abbreviations.
* Soil set numbers as in Soil Bureau Bull. 5.
** Y.G.E. = Yellow-grey earths; Y.B.E. = Yellow-brown earths; Y.B.L. = Yellow-brown loams.

TABLE 1. ACID OXALATE SOLUBLE MOLYBDENUM LEVELS OF SOILS DERIVED FROM VOLCANIC ASH AND INTERMEDIATE AND BASIC IGNEOUS ROCKS

Soil Groups and Parent Materials	Stage of Development †	Soil Sets *	Mean (p.p.m.)	S.D.	No. of samples with Mo level, p.p.m. from 0–.10	.11–.20	.21–.30	.31–.40	>.40	Response of pasture to molybdate topdressing
Recent soils from volcanic ash	-	3, a, 5, a, b, c, e	0.29	0.14	2	2	5	4	3	No information
Soils from Rhyolitic Ash										
1. Yellow-brown pumice soils										
Kaharoa ash	I SU	14–14c, 15, 16, a, b, 17	0.21	0.10	7	27	21	5	2	
Taupo ash	I SU	18 a, b, g, 19–19g, 20, d, e	0.14	0.06	19	33	5	3		No responses have been recorded on soils derived from rhyolitic ash
Taupo ash, early phase, north of Wairoa	I S	18, xa, xg, 19x	0.32	0.10	-	2	6	4	3	
Gisborne ash	I S	21, a, 22, 49, b	0.26	0.09	1	7	9	6	1	
2. Yellow-brown loams										
Whakatane ash	I	51, 51a	0.23	0.16	-	6	2	-	1	
Waihi ash	I LS	54, a, 55, 56a	0.50	0.29	1	2	7	4	18	
Tirau ash	I S	57, 57a, 58	0.57	0.38	-	-	-	7	6	
Soils from Andesitic ash										
1. Yellow-brown Loams										
Mairoa and Maihiihi ash	I LS'	59, a, b, c, 60–60f, 61–61d, 62–62b, 63, 64	0.29	0.19	4	12	18	15	6	
Tongariro ash	S	65, 65b, 65c	0.23	0.13	2	6	6	1	1	No responses recorded on soils derived from andesitic ash
Egmont ash	I S	66, 66d, 67, 67a	0.31	0.12	-	3	11	6	3	
Stratford and Burrell ash	I LS	66b, 67b, 68, 69, 70, 70a, 71	0.29	0.12	2	5	9	3	8	
Water sorted ash (Waitemata suite)	I LS	72, 72a, 72b, 73, 73b	0.19	0.11	6	14	13	3	1	
Rhyolite alluvium and andesitic ash (Horotiu suite)	I	48, 48a, 48b	0.39	0.20	-	3	1	2	4	

2. Brown granular loams and clays										
Water sorted ash (Orere suite)	S	81, 81a,	0.28	0.05	—	—	3	1	—	
Hamilton ash	I–S	82, a, b, 83, b, c, 84 a–d	0.37	0.31	3	15	6	5	13	
Hamilton ash complexes with YBE	—	85a, b, 86–86c	0.16	0.08	8	11	12	1	—	
Soils from Intermediate and Basic Igneous Rocks										
1. Brown granular loams and clays										
Andesitic breccias (Huia suite)	SK–S	HA, YT	0.15	0.04	—	4	—	—	—	No information
Doleritic breccias (Te Kie suite)	SK–M	TE, TU, A, YN, AW, RU, TP	0.11	0.04	9	12	1	—	—	TE, YN, AW, RU—excellent; TU, A, TP no trials
Andesitic flows (Katui suite)	Y–LS	KT, WT, TO, AR	0.14	0.06	4	10	1	—	—	WT excellent; KT, TO, AR no trials but response probable
Alluvium from andesitic basalt (Kohumara suite)	Y–LS	M, KM, P	0.13	0.07	2		1	—	—	M good; KM, P, excellent
2. Complexes with sedimentary rocks										
Andesites and dolerites (Haunga, Kaimaro and Parau suites)	I–LS	OB, PA, DV, HH	0.14	0.07	3	9	1	—	—	OB fair; PA, DV, HH, no information.
3. Red-brown loams										
Basalt flows (Kiripaka suite)	Y–I	OH, WI	0.64	0.40	—	—	—	1	2	No information
	S–M	MC, KE, RT, OK	0.08	0.04	7	3	—	—	—	MC no trials; KE, RT, OK, very slight

† * Code symbols and abbreviations as in previous tables.

TABLE 5. ACID OXALATE SOLUBLE MOLYBDENUM LEVELS OF RECENT, GLEY AND ORGANIC SOILS

Soil Group	Soil Sets	Mean (p.p.m.)	S.D.	No. of samples with mo levels (p.p.m.) from					Responses of pasture to molybdate topdressing
				0–.10	.11–.20	.21–.30	.31–.40	> .40	
Recent soils from alluvium:	1, a, b, c, 2, 2a	0.25	0.15	5	31	24	10	9	Responses do not occur on these soils
Recent soils from alluvium, with peat	2b	0.43	0.28	1	1	1	1	3	
Gley soils with moderate natural drainage:									
Alluvium from volcanic ash	98, a, c, d, 100	0.38	0.19	-	1	1	5	4	Responses do not occur on these soils
Gley soils with poor natural drainage:									
Alluvium from volcanic ash	103, c, d, 104a, b, 105a	0.33	0.14	—	2	5	3	3	
Alluvium from sedimentary rocks	101, b, c, d	0.20	0.17	5	11	7	—	1	
Saline gley soils	111, c, 112	0.41	0.29	-	1	1	1	2	
Organic soils:									
Mellow and intermediate peaty loams	107, 107c-f, 108, a, b, 109, b	0.34	0.25	2	6	3	2	5	
Acid peats	110	0.10	0.08	3	1	1	-	-	One response recorded

TABLE 6. PARTIAL KEY TO CODE LETTERS USED IN NORTH AUCKLAND SOIL SURVEY

Suite	Parent Material	Symbol	Soil Type
Arapohue	Limestone	AU	Arapohue clay
		MT	Motatau clay
Maungaturoto	Lime + mudstone	MO	Maungaturoto clay complex
		MK	Matakohe clay complex
		D	Dairy flat complex
Konoti	Limestone + mudstone + sandstone	KN	Konoti clay loam
Whaka	Calcareous mudstone	RV	Rockvale clay
Pinaki	Sand	PN	Pinaki sand
		WH	Whananaki sand
		MD	Marsden sand
		R	Red Hill sandy loam
		Rs	Red Hill sand
		Rl	Red Hill sandy clay loam
		Rc	Red Hill complex
		TK	Te Kopuru sand
Omu	Claystone, shale etc.	OM	Omu clay loam
		AP	Aponga clay
		MA	Mata clay
		OA	Okaka silty clay and clay
		YK	Waikare clay
		H	Hukerenui silt loam
		WK	Wharekohe silt loam
Omanaia	Sandstone + mudstone	WN	Whirinaki clay loam
		ON	Omanaia clay loam
		AE	Autea clay loam
Puhoi	Banded sandstone	AN	Atuanui clay loam
		OI	Puhoi clay loam
		WR	Whangaripo clay loam
		WA	Warkworth clay
Waiotira	Massive sandstone	WC	White Cone sandy clay
		Y	Waiotira clay loam
		Yr	Waiotira brown clay loam
		Ye	Waiotira light clay
		RP	Riponui clay and sandy clay
		YKs	Waikare clay and sandy clay loam
		Hs	Hukerenui sandy loam
		WKs	Wharekohe sandy loam
Marua	Greywacke	TR	Te Ranga clay loam and stony clay loam
		MR	Marua clay loam
		RA	Rangiora clay, clay loam and silty clay loam
		Hr	Hukerenui silty loam, yellow subsoil phase
Maungarei	Dacite	PE	Parakiore stony clay loam
		PR	Parahaki silt loam and fine sandy loam
Huia	Andesitic breccias and conglomerates	HA	Huia stony clay
		YT	Waitakere clay
Te Kie	Doleritic breccias and tuffs	TE	Te Kie stony loam
		TU	Takitu clay loam
		A	Awapuku clay loam
		YN	Waimatenui clay
		AW	Awarua clay
		RU	Rangiuru clay
		TP	Tinopai sandy loam
Katui	Andesitic flows	KT	Katui clay loam

TABLE 6 (Continued)

Suite	Parent Material	Symbol	Soil Type
		WT	Whatoro clay
		TO	Tutamoe friable clay
		AR	Aranga clay
Kohumara	Alluvium from andesitic basalt	KM	Kohumara clay
		P	Pakotai clay
Haunga	Basalt and sandstone	HH	Hihi clay
Kaimaro	Andesitic basalt and claystone	OB	Onatai clay and loam
Parau	Andesitic tuff and sediments	DV	Dome Valley clay loam
		PA	Parau clay loam
Kiripaka	Basalt flows	OH	Ohaewai silt loam
		WI	Whatitiri clay loam
		MC	Matarau friable clay
		KE	Kerikeri clay loam
		RT	Ruatangata friable clay
		OK	Okaihau gravelly friable clay

COMPARISON OF TRICKLE IRRIGATION AND MEASURED BUCKET WATERING OF GLASSHOUSE TOMATOES

By W. R. Boyce, Horticultural Research Station, Department of Agriculture, Levin

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Summary

Trickle irrigation of glasshouse tomatoes has been compared with measured bucket watering in 1956-57, 1957-58, and 1958-59 on several varieties in an unheated glasshouse at Levin.

Liquid fertiliser was applied through trickle irrigation equipment with each watering in two treatments, and side dressings of solid fertiliser were applied at fortnightly intervals to bucket-watered plots in a third treatment.

The same total quantity of water was applied within near limits by trickle lines and by bucket watering in each season. Quantities applied per plant, at a planting density of 1 plant to 3 sq. ft, varied from 25 to 35 gallons according to the season.

Soil-moisture tensions were maintained as far as possible between 5 and 10 cm under trickle irrigation and between 5 and 25 cm under bucket watering.

There were no differences in the yield of ripe fruit obtained at any stage of picking in any season between plants grown under trickle irrigation and those grown under bucket watering.

Differences in concentrations of liquid fertiliser used in two seasons did not affect yields. The higher concentrations of liquid fertiliser tended to reduce the incidence of blotchy ripening. In the third season a Station-made liquid fertiliser gave as good results as a commercial brand.

Introduction

Trickle irrigation systems for watering glasshouse tomatoes were first introduced into New Zealand about 1953. The manifest advantages of these systems, which are the saving in time and labour involved in hand watering and the ease with which the quantities of water applied can be controlled, have led many growers to install such equipment. Claims have been made that yields are increased and the quality of fruit is improved where plants are grown under trickle irrigation.

Information concerning water and nutrient requirements and the management of glasshouse tomatoes under trickle irrigation has come mainly from sources in Britain where climatic conditions differ considerably from those prevailing in New Zealand.

Practices recommended in Britain call for the use of water-soluble fertilisers based on mixtures of potassium nitrate and ammonium nitrate combined in varying proportions to give $N : K_2O$ ratios of 1 : 3, 1 : 2, 1 : 1, 3 : 1. The maximum solubility of potassium nitrate at 20 lb to 10 gal water places an upper limit on the K_2O content of such solutions at 9.4 lb in 10 gal of water. (Potassium nitrate 13 to 14% N,

42 to 47% K_2O). Nitrogen at 2.8 lb in 10 gal provided by potassium nitrate is supplemented by the addition of ammonium nitrate (34.5% N) to bring the $N : K_2O$ ratios to the ranges listed above. As it is desirable to maintain the potassium content of all such solutions as high as possible, the ratio is altered by varying the amounts of N used. At low temperatures it is difficult to keep potassium nitrate in solution at 20 lb to 10 gal water. More conveniently it may be used at 15 lb to 10 gal but this reduces the K_2O content to a maximum of 7 lb to 10 gal.

Phosphates, for which the indicated requirements of the crop are low, by comparison with nitrogen and potash, are usually supplied in the base dressing. The reasons for omitting phosphates from the trickle feeds are that this nutrient element is absorbed by the soil close to the nozzles and remains there in a restricted zone, and also that phosphates are liable to cause precipitation in the lines and blockage of the nozzles. There is some evidence that phosphates applied in liquid feed can be beneficial at certain stages of growth, but in normal circumstances the plant's requirements can be supplied adequately from base dressings.

It is usual to commence watering with solutions of low $N : K_2O$ ratios i.e., 1 : 3, or 1 : 2, and to increase the proportion of nitrogen as the season progresses, and the intensity of sunlight increases. At the commencement of the season when small quantities of water are applied concentrated solutions are used, for example, 1 part of concentrate to 100 parts of irrigation water, diluting as the season progresses and the amount of water applied increases to 1 part of concentrate to 1000 parts of irrigation water. The main feeds normally used for the greater part of the season comprise equal parts of nitrogen and potash, though Hooper and Young (1957) have advocated an $N : K_2O$ ratio of 1 : 2 for English conditions. With an apparently higher consumption of water by the crop in New Zealand as compared with Britain, it may be advisable to use correspondingly weaker solutions to avoid excessive fertiliser applications.

Investigations were undertaken at Horticultural Research Station, Levin, with the following aims:

1. To compare results obtained using trickle irrigation and liquid fertilisers with those obtained by adopting experimentally controlled practices in hand watering combined with side dressings of dry fertilisers.

2. To ascertain whether practices developed in Britain for the management of glasshouse tomatoes under trickle irrigation could be applied in New Zealand, with particular reference to composition and concentration of liquid-fertiliser solutions and the quantities to be applied.

3. To observe soil reactions in a glasshouse under trickle irrigation, where the history of management is known accurately.

4. To obtain information on water and nutrient requirements of glasshouse tomatoes grown under trickle irrigation.

EXPERIMENTAL

Glasshouse. Plants were set out in August in an unheated aluminium-alloy framed glasshouse 102 x 34 ft. The house runs E.S.E. to W.N.W. and was planted crosswise with a 4ft path down the centre of the house. Rows were 3 ft apart and plants were spaced 1 ft apart in the rows, with the first plant set 1 ft from the walls.

The fruit first ripened in mid November and picking was completed in February.

Soil. The soil type on which the house stands is a mottled phase of Type 76, Levin silt loam (described in the General Survey of Soils of North Island, New Zealand, *N.Z. Soil Bur. Bull n.s. 5*, 1954). This is a yellow-brown loam. The topsoil in the house is a dark-brown silt loam 8 inches deep, overlying a dark-yellow, fine sandy loam with mottlings which indicated that originally drainage was slow in an undisturbed profile. Six feet below the surface coarse gravel and boulders provide free drainage.

A composite sample of the soil taken from a depth of 0 to 6 inches before trial work commenced was analysed as follows:

*C.E.C. me.%	*T.E.C. me.%	Exchangeable cations me.% Ca	Mg	K	Na	% Saturation
18.4	10.3	9.0	.82	.45	.07	56.0

* C.E.C. cation-exchange capacity. T.E.C. total exchangeable cations.

Preparation for Experimental Work. At the outset the soil in the glasshouse was trenched and a compacted subsoil was levelled at a depth of 27 in. below the surface, and then loosened by digging, before the upper layers of soil were restored and levelled above it.

A drain 3 to 4 ft deep was constructed outside the walls of the house and surrounding it. The soil within the house was therefore almost completely isolated from the influence of the water table in surrounding areas. Water applied within the house for flooding and leaching the soil, drained freely and a considerable quantity was taken away by this tile drain.

After the initial preparations had been completed the soil was sterilised with chloropicrin. Similar sterilisation was carried out each year between crops. It was normaly done early in May before soil temperatures fell below 60°F.

Water was applied by sprinkler in sufficient quantity to bring the soil to field capacity to a depth of 3 ft, 3 to 4 weeks before the plants were set out. Tensiometers were placed in the soil at a depth of 6 in. after this heavy watering. The soil was found to be workable when the tensions rose to 8 cm.

Shallow trenches 10 in. wide and 2 to 3 in. deep were then excavated along the rows. The bottoms were made as level as possible

to ensure an even distribution of water in bucket-watered plots. Trenches served to contain the water in bucket-watered plots and to prevent the spread of liquid from trickle nozzles across pathways.

Plants, Planting, and Cultural Operations. The following varieties were used in the first season: Ware Cross, Hertford Cross, Potentate (N.Z.A.) New Zealand approved strain, Potentate (L.) a strain supplied by the Glasshouse Crops Research Institute Littlehampton, Potentate (N.Z.C.) a New Zealand commercial strain, and Moneymaker. Two varieties only, Ware Cross and Potentate (N.Z.A.), were grown in the second and third seasons.

Plants were raised from seed sown in June and pricked out into boxes in the first two seasons, and into peat and wood fibre pots in the third.

Kerosene heating was used in the first two seasons in the propagating house. Temperatures could not be controlled exactly. In the third season electric heating was installed and accurate temperature control was possible. The seed was germinated at 60° to 62°F. Temperatures were lowered to 58°F as the seed leaves emerged and from the 8th to the 12th night temperatures were reduced to 53° to 54°F. Temperatures of 60°F were then resumed until the cold check treatment was repeated 10 days later.

Plants were set out while soil and air temperatures were still rather low but rising with lengthening daylight hours and increasing intensity of sunshine. There was no evident need to restrain plants in the early stages of growth.

Orthodox practices were followed in training the plants. Single stems were taken up to the wires. Usually 8 to 10 flowering trusses were obtained below the wires. Plants were permitted to produce 10 trusses and were then stopped. Lateral growths were removed at frequent intervals. Foliage removal was delayed as long as possible, and often it was carried out only to assist in bringing *Botrytis* infection under control.

Hormone fruit-setting sprays were applied regularly until fruit commenced to set freely. At least 2 sprays were applied to each flowering truss at intervals. Ferbam fungicide was included with the hormone sprays for *Botrytis* control. No damping down was practised.

Therapeutant sprays were applied every 10 to 14 days with a paint spray gun for disease control. *Botrytis* was the only disease to cause concern during the course of these trials. No serious losses of fruit were sustained. The disease was brought under control by more frequent spraying, by the use of alternative fungicides, by routine practices such as the removal of damaged leaves and older foliage, and by the use of ventilation. *Cladosporium* leaf mould caused very little trouble. A very few plants were lost with *Verticillium* wilt and *Sclerotinia* rot.

Fruit was picked twice weekly in 1956-57 season and once a week in the other two seasons.

At the end of each season's trial when picking was completed and the plants had been removed, the soil was first flooded through trickle lines (lines were laid along hand-watered plots for this purpose) and then by means of sprinklers. Several copious applications of water were made at intervals in order to leach residual nutrients. After the 1957-58 season's trial 29,000 gallons of water were applied.

Wheat straw was worked into the soil after the 1956-57 season's crop at the rate of 17 tons per acre, with 84 lb of sulphate of ammonia for each ton of straw. At the end of 1957-58 season a maize cover crop was grown and cultivated in.

Soil Testing. Soil samples were taken for analysis before planting, during the growing season, and at the end of the season. Samples were taken also after flooding had been carried out. Applications of lime and base fertiliser were made in accordance with the results of the soil analyses. The quantity of phosphate applied in the base dressing was increased in the third season because of suspected phosphate deficiency in part of the house. In this area the growth of plants was retarded and soil analysis showed fairly low levels of available phosphate. At the end of the season no difference in yield could be detected between the retarded rows and plants elsewhere in the house.

There has been a marked fall in pH values in the house from season to season. Lime was applied at the rate of 4 tons per acre after the 1957-58 crop, but this was not sufficient to maintain pH values in the following season. In December 1958 pH 5.0 was recorded in trickle-irrigated plots and pH 4.7 in bucket-watered plots. By February 1959 these pH values had fallen to 4.4.

Analyses of drainage water were made during the course of post-harvest flooding after the 1957-58 crop.

Equipment. Trickle-irrigation equipment installed consisted of $\frac{3}{4}$ in. rubber mains hose with $\frac{3}{8}$ in. trickle lines leading from the mains. Metal nozzles (one per plant) were fitted to the trickle lines. These nozzles were designed to deliver 2 pints of water per hour under a pressure head of 6 ft of water in the lines. Variable-range diluter heads through which the proportion of a concentrated fertiliser to irrigation water could be varied from 1 : 50 to 1 : 1000 were fitted to clear-glass bottles of 18-pint capacity which held the concentrated fertiliser solution. Water is bypassed in the diluter head to displace an equivalent volume of fertiliser solution into the irrigation water flowing to the mains and trickle lines.

Sleeve adaptors were inserted in the diluter heads for the small deliveries required in these experiments. For 360 plants in each trickle-watering treatment, with applications of 1 to 3 pints per plant, only 45 to 135 gallons of water were needed at each watering. With such small deliveries the performance of the diluter heads was sometimes erratic. Water meters were installed on each supply tap. The total quantity of water to be applied was accurately determined by meter readings. From the quantities of water applied and the indicated dilution rates, the theoretical consumption of concentrate could be

calculated. The concentrate bottles were graduated and from the level of the interface between the dye-coloured concentrate and the supernatant water the actual consumption of concentrate could be gauged approximately. As the interface was not sharply defined but became a diffused zone 2 inches deep as the concentrate was used up, it was not an exact measure. The procedure adopted was to calculate from meter readings and the indicated dilution rates, when the concentrates should have been consumed. The concentrate bottles were then removed from the diluter heads and the actual consumption of concentrate was read from the graduated scale on the bottles. These bottle readings were taken to represent the quantity of concentrated solution actually applied and were used in calculating the nutrient applications shown in Table 3. The experimental error obviously could be fairly large, but this applied mainly in cases where only small quantities of a particular solution were applied. Where the full charge of concentrate was used quickly the observed and calculated deliveries corresponded fairly closely.

Deliveries from nozzles were checked on a number of occasions and found to vary up to 20% between some individual nozzles, even when these were running freely. The majority were less variable. As each individual plant would be absorbing water applied from at least 3 nozzles, minor differences in delivery were not important, and the deliveries to each plant tended to even out over the 15 nozzles on each trickle line. Regular inspections were necessary to detect blocked and sluggish nozzles. In the second season the trickle lines were raised off the ground on wire loops. Blocked nozzles were detected more easily and blockages occurred less frequently.

Water was applied to bucket-watered plots by means of graduated plastic buckets which conveniently held 15 pints. One bucket per row supplied 1 pint per plant.

Gallenkamp porous-pot soil-moisture tensiometers were used to measure soil-moisture tensions. One pilot tensiometer was used in each treatment. An extra one was employed for one trickle treatment, while a fifth instrument was retained as a spare and was used if necessary to check the performance of the other instruments. The three pilot tensiometers for each watering treatment were placed in corresponding positions in each plot, usually between the third and fourth plant from the centre path of the house. Tensiometers were placed vertically in the plant row, midway between plants 12 in. apart, with the mid point of the porous pot 6 in. below the surface. Readings were taken at 8 a.m. and 4 p.m. daily.

The equipment used to apply therapeutants with a paint spray gun has been described elsewhere (Boyce *et al.* 1959).

Design of Trials

In the 1956-57 trial, single-row plots were used in a 3 × 6 factorial experiment (three watering treatments and six varieties) with a randomised-block design. There were 54 treated rows of 15 plants, 27 on each side of the house, with 3 guard rows at each end, 12 in all.

At the conclusion of this trial, excavations showed that roots of the vigorous variety Ware Cross had grown across the 3-ft pathway separating adjacent plots, and these roots were ramifying in the trenches of adjacent plots under different watering treatments. Though it seems unlikely that the results of the trial were affected, the possibility of some interference between treatments could not be discounted.

For the 1957-58 and 1958-59 trials 3-row plots were used, and the number of varieties was reduced to two, in a 3 × 2 factorial experiment in which a similar randomised-block design was used. This enabled the results to be analysed using the centre rows only and a comparison of such an analysis to be made with that for the full 3-row plots. There was no positive indication that interference had occurred, although in view of the very small differences in yield among the treatments, it would have been almost impossible to detect it.

Treatments were re-randomised each year. Copious flooding, cover cropping, and intensive cultivation of the soil was used to minimise the carry over of residual effects from one season to the next.

Recordings

Maximum and minimum air temperatures, soil temperatures, humidity, and soil moisture tensions, were recorded at 8 a.m. and 4 p.m. daily. Maximum-minimum temperatures were taken at 3 positions —at the ends and in the middle of the house. The thermometers were suspended immediately above young plants and raised as the plants grew until in their final positions the thermometers were 5 ft above ground level. Soil thermometers of a remote dial reading type were installed in two positions and temperatures at 1–6 inches depth were recorded. A sling psychrometer was used to determine humidities.

The quantity of water or nutrient solution applied was recorded at each watering in pints per plant and gallons per treatment. On trickle treatments, water-meter readings gave accurate records of the quantity of water applied. On bucket-watered plots, measured applications on a pints-per-plant basis enabled the quantity of water applied per treatment to be calculated. The composition and concentrations of nutrient solution applied at each watering were also recorded as was the quantity of concentrated fertiliser consumed at each change or replacement of concentrate in the diluter bottles.

Hours of bright sunshine were recorded from a Campbell-Stokes recorder in a meteorological station sited 60 yd from the glasshouse.

The percentage of blotchy ripened fruit is shown as a percentage number in the first two seasons and as a percentage by weight in the third season. Where the incidence of blotchy ripening was very low, a few fruit per row, counting was more satisfactory than weighing.

Fertiliser Applications

Base Dressings. Applied to total floor area of the house, 3468 sq. ft.

	1956-57, 57-58	1958-59
Serpentine superphosphate	175 lb (1 ton/ac. approx.)	350 lb (2 ton/ac. approx.)
Bonedust	175 lb (1 ton/ac. approx.)	200 lb (1⅐ ton/ac. approx.)
Sulphate of potash	88 lb (½ ton/ac. approx.)	88 lb (½ ton/ac. approx.)
Sodium molybdate	3¼ oz (2½ lb/ac.)	0

Serpentine superphosphate, 15% P_2O_5;
Bonedust 3% N, 20% P_2O_5.

Side Dressings. Ten side dressings of the following solid fertiliser mixture were applied at 14-day intervals at the rate of ½ oz per plant to bucket-watered plots. The fertiliser was evenly distributed in a band down the rows.

	N	K_2O	
Dried blood	12%	—	3 parts
Sulphate of ammonia	20.5%	—	1 part
Sulphate of potash	—	48%	1 part

The 11.3% N to 9.6% K_2O ratio of this side-dressing mixture is similar in composition to the trickle-fertiliser solution mainly used, which has 9.7% N to 8.3% K_2O.

Liquid Fertiliser Solutions Used in Trickle-irrigation Treatments. In accordance with accepted practice the composition of liquid-fertiliser solutions is expressed as pounds of nutrient, N, P_2O_5, and K_2O, contained in 10 gal or 100 lb of water, or as a percentage, pounds of nutrient to 100 lb of water. This indicates a higher percentage composition than when nutrient is shown as weight to weight of solution. For instance, 28.6 lb of ammonium nitrate (35% N) contains 10 lb of nitrogen. Therefore 28.6 lb of ammonium nitrate dissolved in 10 gal water gives a liquid-fertiliser solution with 10% nitrogen, if this convention is used, whereas on a weight of nutrient to weight of solution basis the percentage is only $\frac{10}{128.6} \times \frac{100}{1}$ or 7.8%.

A commercial brand of liquid fertiliser was used in the first two seasons. From a concentrate containing 20.0 parts N and 7.0 K_2O, two standard solutions containing approximately 4.5 N and 9.0 K_2O, and 9.7 N and 8.3 K_2O, were prepared by the addition of potassium nitrate solution containing 2.8 N and 9.4 K_2O. These fertiliser solutions were used at two different concentration ranges as shown below.

Treatment	1956-57		1957-58	
	Trickle X	Trickle ½ X	Trickle X	Trickle 2 X
	1 : 150	1 : 300	1 : 300	1 : 150
	1 : 200	1 : 400	1 : 400	1 : 200
	1 : 300	1 : 600		
	1 : 400	1 : 800		
	1 : 500	1 : 1000		

In the 1958-59 season the commercial brand of liquid fertiliser was compared with solutions of equivalent N and K_2O content made up from potassium nitrate (13.82% N and 46.94% K_2O) and ammonium

TABLE 1. COMPOSITION, CONCENTRATION AND QUANTITY OF LIQUID FEEDS APPLIED WEEKLY, IN TWO TRICKLE TREATMENTS, DURING FOUR-WEEKLY PERIODS IN EACH MONTH FROM SEPTEMBER TO FEBRUARY, 1956-57, 1957-58, 1958-59

Month	Week	Composition and Concentration of Liquid Feeds Applied												Quantity Applied per Plant Total per Week in Pints		
		1956-57				1957-58				1958-59				1956-57	1957-58	1958-59
		N : K_2O		TX	$T\frac{1}{2}X$	N : K_2O		TX	T2X	N : K_2O		TX	T.HRS	Per Week	Per Week	Per Week
Sep.	1	—	—	—	—	4.5	9.0	1 : 300	1 : 150	4.5	9.0	1 : 150	1 : 150	—	2.0	4.5
	2	4.5	9.0	1 : 150	1 : 300	,,	,,	,,	,,	,,	,,	,,	,,	3.5	5.0	6.5
	3	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	7.0	3.5	8.0
	4	,,	,,	1 : 200	1 : 400	,,	,,	,,	,,	,,	,,	,,	,,	6.5	4.5	8.0
Oct.	1	9.7	8.3	,,	,,	,,	,,	1 : 400	1 : 200	9.7	8.3	1 : 200	1 : 200	6.0	5.0	11.0
	2	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	6.5	6.0	4.0
	3	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	8.5	9.5	9.5
	4	* ,,	,,	1 : 300	1 : 600	9.7	8.3	,,	,,	,,	,,	,,	,,	9.0	8.0	8.5
Nov.	1	,,	,,	,,	,,	,,	,,	,,	,,	* ,,	,,	,,	,,	8.5	7.0	11.5
	2	,,	,,	,,	,,	,,	,,	,,	,,	* ,,	,,	,,	,,	9.0	8.0	10.5
	3	,,	,,	,,	,,	,,	,,	1 : 300	1 : 150	,,	,,	,,	,,	13.0	7.0	12.0
	4	* ,,	,,	,,	,,	,,	,,	,,	,,	* ,,	,,	1 : 300	1 : 300	12.5	6.0	15.5
Dec.	1	,,	,,	,,	,,	,,	,,	1 : 400	1 : 200	4.5	9.0	,,	,,	12.0	9.5	13.5
	2	,,	,,	,,	,,	,,	,,	,,	,,	9.7	8.3	,,	,,	11.5	7.5	12.5
	3	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	12.5	8.5	10.5
	4	* ,,	,,	1 : 400	1 : 800	* ,,	,,	,,	,,	,,	,,	,,	,,	13.0	7.5	10.0
Jan.	1	,,	,,	,,	,,	* ,,	,,	,,	,,	,,	,,	,,	,,	13.5	8.0	12.0
	2	,,	,,	,,	,,	* ,,	,,	,,	,,	,,	,,	1 : 400	1 : 400	16.5	11.0	11.5
	3	* ,,	,,	1 : 500	1 : 1000	* ,,	,,	,,	,,	,,	,,	,,	,,	19.5	8.5	16.5
	4	,,	,,	,,	,,	20.0	7.0	,,	,,	,,	,,	,,	,,	21.0	11.0	14.0
Feb.	1	* ,,	,,	,,	,,	9.7	8.3	,,	,,	,,	,,	,,	,,	20.0	11.0	—
	2	,,	,,	,,	,,	* ,,	,,	,,	,,	,,	,,	,,	,,	—	—	—
	3	—	—	—	—	* ,,	,,	,,	,,	,,	,,	,,	,,	—	—	—

* Booster feeds with a high N content 20 N : 7 K_2O were given for one or two applications at times shown.

nitrate (34.5% N), and used at the same concentration ranges. These solutions referred to as Trickle H.R.S. were made up as follows:

N (%)	K_2O (%)	$(NH_4)NO_3$ (lb)	KNO_3 (lb)	Water (gal)
4.5	9.0	5.36	19.17	10
9.7	8.3	21.01	17.70	10
20.0	7.0	52.00	14.91	10

The concentration ranges used were 1 : 150, 1 : 200, 1 : 300 and 1 : 400.

TABLE 2. NUTRIENTS APPLIED IN BASE DRESSINGS, BALL WATERINGS, SIDE DRESSINGS AND TRICKLE IRRIGATION IN POUNDS TO 270 PLANTS OR 810 SQ. FT

1956-57	Bucket Watering			Trickle X			Trickle ½ X		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Base dressing	1.2	14.3	9.9	1.2	14.3	9.9	1.2	14.3	9.9
Side dressing	9.5	0.0	8.1	0.0	0.0	0.0	0.0	0.0	0.0
Trickle	0.0	0.0	0.0	22.4	0.0	18.8	11.8	0.0	9.8
Totals/810 sq. ft	10.7	14.3	18.0	23.6	14.3	28.7	13.0	14.3	19.7
1957-58	**Bucket Watering**			**Trickle X**			**Trickle 2 X**		
Base dressing	1.2	14.3	9.9	1.2	14.3	9.9	1.2	14.3	9.9
Side dressing	9.5	0.0	8.1	0.0	0.0	0.0	0.0	0.0	0.0
Trickle	0.0	0.0	0.0	15.7	0.0	12.7	29.6	0.0	23.6
Totals/810 sq. ft	10.7	14.3	18.0	16.9	14.3	22.6	30.8	14.3	33.5
1958-59	**Bucket Watering**			**Trickle X**			**Trickle H.R.S.**		
Base dressing	1.4	21.6	9.9	1.4	21.6	9.9	1.4	21.6	9.9
Side dressing	9.5	0.0	8.1	0.0	0.0	0.0	0.0	0.0	0.0
Ball watering	0.2	Trace	0.3	0.2	Trace	0.3	0.2	Trace	0.3
Trickle	0.0	0.0	0.0	25.9	0.0	24.6	28.2	0.0	25.4
Totals/810 sq. ft	11.1	21.6	18.3	27.5	21.6	34.8	29.8	21.6	35.6

ESTIMATED NUTRIENT REMOVALS FOR AN 80-TON CROP
(AFTER OWEN 1929, AND KIDSON *et al.* 1953)

	Per 810 sq. ft (lb)			Per acre (100 lb)			
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	
Owen (1929)	13	2	26	6.8	1.2	14.0	Includes all foliage
Kidson (1953)	6	3	14	3.1	1.7	7.7	Omits prunings

TABLE 3. COMPARISON OF WEIGHTS OF RIPE MARKETABLE FRUIT AND PERCENTAGE OF BLOTCHY-RIPENED FRUIT OBTAINED FROM BUCKET-WATERED AND FROM TRICKLE-IRRIGATED TOMATOES IN THREE SEASONS' TRIALS

Watering Systems	Weight of Ripe Marketable Fruit					Proportion of Blotchy-ripened Fruit*	
	Lb per plant per 3 sq. ft			lb/1000 sq. ft	Tons/ acre		
	To Nov. 30	To Dec. 31	Total	Total	Total	% By Number	
1956-57							
Bucket	.67 a A	5.53 a A	15.5 a A	5169	100.5	3.5	a A
Trickle X	.67 a A	5.45 a A	15.6 a A	5209	101.3	2.0	b B
Trickle ½ X	.67 a A	5.48 a A	15.5 a A	5163	100.4	3.5	a A
1957-58							
Bucket	Not anal	3.50 a A	12.0 a A	4000	77.8	2.0	a A
Trickle X	Not anal.	3.50 a A	12.0 a A	4000	77.8	1.7	ab A
Trickle 2 X	Not anal.	3.60 a A	11.8 a A	3933	76.5	1.3	b A
1958-59						% By Weight	
Bucket	1.29 a A	6.24 a A	10.7 a A	3570	69.4	7.4	a A
Trickle X	1.32 a A	5.68 a A	10.3 a A	3440	66.9	5.9	a A
Trickle HRS	1.32 a A	5.74 a A	10.4 a A	3460	67.3	5.8	a A

* Blotchy-ripened fruit is shown as a percentage of total ripe fruit; that is ripe marketable fruit plus blotchy-ripened fruit.
In 1956-57 and 1957-58 season blotchy-ripened fruit was counted and the number of such fruit is shown as a percentage of the total number of ripe fruit. In 1958-59 season blotchy-ripened fruit was weighed and shown as percentage by weight.
Significance tests (Duncan 1955). Weights with a letter in common do not differ significantly. Small letters represent tests of significance at 5% level, capitals tests at 1% level.

Details of composition, concentration and quantity of liquid fertilisers applied are shown in Table 1.

The quantities of nutrients applied as N, P_2O_5, and K_2O are shown in Table 2.

RESULTS

Yields obtained over three seasons in terms of ripe marketable fruit are shown in Table 3.

Watering Systems. The results show clearly that in these trials there were no differences in yield at any stage of cropping between measured bucket watering and trickle irrigation, in any season. The yields from the three treatments were remarkably close, 15.5, 15.6, and 15.5 lb of fruit per plant in 1956-57, 12.0, 12.0, and 11.8 lb in 1957-58, and 10.7, 10.3, and 10.4 lb in 1958-59, for bucket watered, first trickle and second trickle treatments, in that order.

There were no differences between the two trickle treatments. In the first season the proprietary liquid fertiliser used at a range of concentrations TX, and at half these concentrations T½X, gave the same

yield. A range of concentrations TX, and double these concentrations T2X, in the second season gave the same yield. In the third season a liquid fertiliser made up from ammonium nitrate and potassium nitrate to give solutions of similar composition to the proprietary fertiliser and used at the same concentrations as the proprietary fertiliser, gave just as good results.

Trickle irrigation used at the higher concentration ranges in the first two seasons' trials reduced the quantity of blotchy ripened fruit, by comparison with the amount obtained with bucket watering. Although the percentage of affected fruit was small, the reduction was significant. There were no significant differences between the two trickle treatments, nor between the trickle treatment at the lower concentration range and bucket watering.

In other respects the quality of fruit assessed by scores for shape and colour did not differ between trickle-irrigated and bucket-watered plots.

Varieties. The performances of the varieties used in these trials will be described elsewhere.

Varieties and Watering Systems. There were no consistent significant interactions between varieties and watering systems in yield or quality of fruit. In the 1957-58 trial when total yield of fruit, including green fruit picked at the end of the season, was considered, Ware Cross gave a higher yield under bucket watering, and Potentate did better under trickle irrigation. The difference was significant only at the 5% level.

Applications of Water and Trickle Solutions. The total quantities of water applied by bucket and liquid feeds by trickle irrigation were about the same within each season. Details are shown in Table 4 In round figures 35 gallons of water were applied by bucket to each plant in 1956-57 season, and 34 gallons by trickle. Amounts for the next two seasons were 27 and 25, and 30 and 29 gallons for bucket and trickle respectively. This close correspondence was not accidental. Although tensiometers were used independently in the trickle- and bucket-watered systems to indicate when water should be applied, it was found that the sum of the quantities applied frequently and in small amounts by trickle to maintain low-tension water regimes could be used to determine the larger quantities required at longer intervals under the higher-tension regimes of bucket watering. Thus the quantities applied balanced out almost exactly in the different systems, at the end of the seasons.

The greater quantity of water applied in the first season could be attributed to the many more hours of sunshine recorded during the growing season in that year.

Table 4 shows that water was applied by bucket 47, 62, and 47 times, and by trickle 156, 154, and 155 times, over growing seasons of 177, 204, and 179 days, from planting to final harvest, in the three seasons consecutively.

TABLE 4. TOTAL APPLICATIONS OF WATER BY BUCKET AND LIQUID FERTILISER BY TRICKLE IN 1956-57, 1957-58, 1958-59
(per plant at 3 ft × 1 ft spacing)

Season	Days from Planting to Final Pick	Hours Sunshine	Number of Days Water or Nutrient Applied		Water or Nutrient Applied (Pints)	
			Bucket	Trickle	Bucket	Trickle
1956-57	177	1232	47	156	282.0	275.5
1957-58	204	990	62	154	217.0	201.0
1958-59	179	1056	47	155	240.5	235.0

Monthly applications in pints per plant, averaged over three seasons for the 5 complete months from September to January, were 20, 40, 48, 48, and 61 by bucket, and 22, 34, 44, 48, and 60 for corresponding months by trickle.

The quantities applied each week by trickle feeds for the same 5-monthly periods from September to January are shown on the right hand side of Table 1. The range of amounts given at each feed was $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, or 3 pints per plant, increasing as the seasons progressed. Applications of $\frac{1}{2}$ pint were very seldom used. Applications through the two trickle systems were always made at the same time and the same quantities were used.

Plants always appeared to be adequately supplied with water. Only occasionally were signs of wilting noted at the tops on very hot days. No overhead damping was done during the course of these trials.

Nutrients Applied. Rates of application of nutrients N, P_2O_5, and K_2O in solid fertiliser dressings and liquid fertiliser feeds are compared for three watering systems over three seasons' trials in Table 2.

Nutrients applied in base dressings before planting were the same for all treatments. In the third season the amount of phosphate in the base dressing was increased to overcome indications of phosphate deficiency noted in the early part of the previous season. For the same reason ball watering was carried out for a period after planting in the 1958-59 season, with a solution containing small amounts of phosphate.

Nutrients supplied as side dressings of solid fertiliser to the bucket-watered treatments were constant over the three seasons, except for small amounts of nutrients used in ball watering in the third season.

Applications of nutrients to bucket-watered plots corresponded closely to the total quantities applied in the lower-concentration trickle treatments (i.e. to Trickle $\frac{1}{2}$X) in 1956-57, and to Trickle X in 1957-58. Rates in the other trickle treatments were about double.

Details of the feeds used as the seasons progressed are shown in Table 1. For clarity in presentation the data are confined to four weekly periods in the months from September to January and there has been a slight adjustment of dates where alterations occurred in the

composition or concentration of solutions applied, on the 29th, 30th, or 31st day of a month.

The different rates of application of nutrients had no effect whatsoever on yield.

Soil Analyses. These indicated that satisfactory levels of potassium and phosphorus were maintained in the course of the trials. Calcium levels fluctuated and pH values had fallen to the very low levels of 4.4 and 4.5 at the end of this series of trials, in spite of generous liming treatments. Low pH occurred in both bucket-watered and trickle-irrigated treatments.

Soil samples taken early in March 1958 at the conclusion of the 1957-58 trial were analysed as follows:

	C.E.C.* me.%	T.E.C.* me.%	Exchangeable Cations (me.%)				
			Ca	Mg	K	Na	pH
Hand watered	18.7	6.6	5.1	0.27	0.89	0.32	4.6
Normal trickle	17.7	6.8	4.9	0.29	1.07	0.49	4.4
Double trickle	17.6	5.5	3.5	0.24	1.43	0.32	4.3

* Cation-exchange capacity * Total exchangeable cations

No signs of magnesium deficiency were noticed in the crops. Although soil analysis levels were low, leaf samples taken at the same time showed levels of 0.6% Mg, an adequate figure (McNaught 1952).

Analyses for soluble salts usually showed higher levels under trickle-irrigation treatments. No observations could be made on cumulative effects as the positions of treatments were altered from season to season.

Flooding with copious applications of water at the end of each season's trial was applied as a precaution against accumulation of salts. At the end of the 1957-58 trial, 29,000 gal water were applied for this purpose. Samples of drainage water were taken for analysis after 12,000 gal had been applied and again after 20,000 gal. Results are shown as grammes of ion per litre of solution.

The pH of the first and second samples were 6.1 and 5.6 and the conductivities 1.37 and 1.17 millimhos/cm respectively.

High nitrate was to be expected on account of ammonium and potassium nitrates contained in trickle feeds. Small amounts of ammonium ion indicated that either it had been converted to nitrate or had exchanged with other cations. The level of potassium by comparison with sodium, magnesium, and calcium was unexpectedly low in view of heavy dressings of potash in the base dressings and trickle feeds.

	Cations (g/l)					Anions (g/l)			
	K	Na	NH4	Ca	Mg	NO_3	Cl	SO4	PO4
After 12,000 gal	.0201	.0301	.0035	.147	.0205	.643	.051	.082	N.D.
After 20,000 gal	.0201	.0243	.0053	.120	.0185	.503	.052	.097	N.D.

N.D. = Not detected (less than .001 g per litre).

DISCUSSION

Good crops of tomatoes were raised in the course of these trials. Requirements for experimental work were fulfilled under management which could be applied in good commercial growing practices.

Ratio of Nitrogen to Potassium. Trickle feeds with a solution containing 4.5 parts nitrogen (N) to 9 parts potash (K_2O) were used for the first 3 to 7 weeks after regular trickle feeding commenced in September. The solution ratio was then changed to one with 9.7 N to 8.3 K_2O, which was used for the remaining 22 weeks. Occasionally a solution with a much higher nitrogen content 20 N to 7 K_2O was applied for one or two feeds, and on one occasion it was used for a longer period when the growth and appearance of the plants seemed to indicate that more nitrogen was required. The use of a solution with an approximate N : K_2O ratio of 2 : 2 as the main feed used over the greater part of the season differs from recommendations published by Hooper and Young (1957a, b) for the guidance of advisory officers in Britain, in which the main feeds recommended for the greater part of the growing season contain N : K_2O in a 1 : 2 ratio, equivalent to the early season feeds used in these trials. For unheated houses planted in August or later in New Zealand the use of a feed with the higher nitrogen content as the main feed, appeared to be warranted by the greater intensity of sunshine prevailing in this country.

Concentration of Fertilisers. The range of concentrations used varied from 1 part of fertiliser solution to 150 parts of irrigation water (1 : 150) to 1 : 500, in one trickle treatment, and from 1 : 300 to 1 : 1000 in the other during the first season in which the sunshine hours and the amounts of water applied were greatest. In the second and third seasons much narrower ranges were used, 1 : 150 to 1 : 200, and 1 · 300 to 1 : 400 in 1957-58, and 1 : 150 to 1 : 400 in both treatments in 1958-59. These concentrations fall within recommended ranges. The use of very concentrated solutions was avoided. The more dilute solutions may be required at Levin in sunny seasons when the consumption of water is higher, and this probably applies to districts with a high number of sunshine hours and to the northern parts of North Island.

Composition of Fertilisers. In the bucket-watered treatments the fertiliser mixture used in side dressings was made up from dried blood, ammonium sulphate, and potassium sulphate, materials used in normal commercial practice. The N : K_2O ratio 11.3 : 9.6 was designedly

similar to that in the main trickle feed used. Nutrient applications by means of side dressings were more sparing than those applied by trickle feeds, and were given at 14-day intervals. This did not affect yields adversely.

The trickle-feed solution prepared from potassium nitrate and ammonium nitrate gave as good results as the proprietary fertiliser. This aspect of trickle feeding has been investigated more fully in another trial.

Application of Water and Trickle Solutions. Soil-moisture tensiometers were used to indicate when water or liquid feed was to be applied. The tensions were 25 to 30 cm for bucket-watered treatments and 10 cm for trickle treatments. Within these tension ranges the tensiometers functioned satisfactorily and generally appeared to be quite reliable.

As water or trickle feeds were regularly applied at 8 a.m., weather conditions on the preceding day and the outlook for the day ahead were taken into account in judging the quantity of water to be applied. Applications made in trickle treatments in these trials were mainly from 1 to 3 pints. Half pints were seldom used and 3 pints were not exceeded.

For the greater part of the season, October to January, trickle feeds were given every day and bucket waterings every third or fourth day. The quantities to be applied by bucket were assessed from the trickle applications on the intervening days, and in each trial the amount of water applied in bucket and trickle systems balanced closely.

As almost identical yields were obtained under all watering treatments in each trial and the yields were good, it is reasonable to assume that water and nutrients supplied were adequate for crop requirements in both trickle- and bucket-watered plots.

These trials have demonstrated that under the conditions described just as good results can be obtained from the application of water and side dressings of solid fertiliser, as from the use of liquid fertilisers through trickle-irrigation equipment. This will hold true only where the application of water by hose or bucket, and nutrients by side dressing can be timed and measured to meet plant requirements adequately and accurately. Trickle feeding with liquid fertilisers has brought these objectives within reach of the grower. Trickle irrigation has other manifest advantages. It is less laborious and may save considerable time and labour, which can be devoted to other work essential for better management, or it may enable a grower to increase his area under glass and secure a better living. These merits explain the ready acceptance of trickle irrigation by many growers. However, it is possible that an inexpensive system of watering could be devised which would enable measured amounts of water to be applied, say twice weekly, at the turn of a tap, in such a way that side dressings of solid fertiliser could be used. This would combine the labour-saving advantages of trickle irrigation with accurate measurement and delivery of water applied and with the use of less costly and more readily available fertilisers.

The marked fall in yield after the first season has not been fully explained. It is well known that the first crop taken from a newly erected glasshouse is frequently better than subsequent crops. That the yield of a glasshouse tomato crop is affected by the hours of bright sunshine it receives during the growing season is also known. The total hours of sunshine received in the first season was much greater than in the second and third. But these factors alone do not seem to be sufficient to explain a fall in yield from 16.7 lb per plant to 12.0 lb, and to 10.5 lb, the average yield obtained from the two varieties Ware Cross and Potentate (N.Z.A.), in successive trials. There has been no build up of soil-borne disease. The soil has been sterilised each year. Nutrient levels as shown by soil analyses were maintained, although soil acidity increased to an extreme acidity of pH 4.4 which could adversely affect nutrient availability. Organic matter (straw and a maize cover crop) was worked into the soil to maintain its condition.

There was evidence that higher concentrations of trickle feeds reduced the incidence of blotchy ripening. As the incidence of this disorder was low, the value and effectiveness of increasing the concentration of liquid feeds as a preventive measure could not be assessed. The reduction was only by comparison with bucket watering and the additional fertiliser used brought no compensating increase in yield.

The varieties used in this trial responded well to the management and treatments applied. With one exception, there were no interactions between varieties and watering treatments. Nor was there any apparent need during the growing of the crops to apply differential watering treatments to suit particular varieties.

CONCLUSIONS

In trials extending over three seasons in an unheated glasshouse at Levin, trickle irrigation of glasshouse tomatoes with liquid fertilisers did not produce better results than measured bucket watering with side dressings of fertiliser.

The main advantage of trickle irrigation for glasshouse tomatoes is that it enables precise control of water and fertiliser applications to glasshouse tomato crops to be attained more easily than with conventional methods of hose or bucket watering.

Practices developed in Great Britain for the management of glasshouse tomatoes under trickle irrigation were tested in these trials. The compositions and concentrations of the liquid fertilisers used, and the water regimes applied were based on the findings of research carried out in Britain and on recommendations of firms supplying fertilisers and irrigation equipment in that country. Certain modifications, such as the use of solutions of higher nitrogen content in the main feeds, were made because of the known climatic differences between the two countries. The methods used at Levin in these experiments, judged by the results obtained, were successful, and should serve as a basis for advisory work in this country.

More information is required on the nutrient needs of glasshouse tomatoes grown under trickle irrigation on New Zealand soils and under

New Zealand conditions before the most suitable fertiliser applications in trickle feeds can be defined.

Provided that base dressings of phosphate and potash fertilisers and lime are used in accordance with advice based on soil analyses, the quantities of nitrogen and potash applied to the growing crop can vary considerably without effect on yield, and the means of applying these nutrients either by side dressings or as trickle fertilisers are unimportant.

The water requirements of tomatoes at Levin, as indicated by an average taken over 3 seasons, is about 30 gal per plant, at a planting density of 1 plant to 3 sq. ft of planted glasshouse space. This is equivalent to 435,600 gal of irrigation water per acre, or 19 inches. A similar requirement has been reported for a heated house at Palmerston North—some 35 miles distant (Hockey 1957). These figures provide a datum for the Manawatu district and a guide to the water requirements of glasshouse tomatoes in other localities.

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A COMPARISON OF PENMAN'S AND THORNTHWAITE'S METHOD OF DETERMINING SOIL MOISTURE DEFICITS

By P. D. Fitzgerald and D. S. Rickard, Winchmore Irrigation Research Station, Ashburton

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Summary

Thornthwaite's and Penman's method of calculating transpiration rates have been used to estimate soil-moisture deficits over the irrigation intervals of a field experiment. These calculated deficits were compared with the deficits measured prior to each irrigation by the gravimetric technique. Values of Penman's factor 'f', evaluated from the irrigation intervals of a second field experiment were found to differ from those used by Penman for England.

Both methods are equally reliable for estimating soil-moisture deficits and could be used under local conditions for the timing of irrigations, within the range at which transpiration could be assumed to be at the potential rate. The correlations between measured and calculated deficits were the same in both cases, + 0.57.

Introduction

The two most widely known methods of determining transpiration from standard meteorological observations are due to Penman (1948) and Thornthwaite (1948). The two methods differ widely in their approach and ease of calculation. Penman's method is based on a partly empirical reconciliation of aerodynamic and heat-balance approaches and requires a knowledge of mean air temperature, relative humidity, sunshine hours, and wind miles. The computations are more involved than those used in Thornthwaite's method, and because short-wave radiation from the sun and sky is estimated from sunshine hours by an empirical relation, which is certainly not true for periods of less than a week, it cannot be used on a daily basis. Thornthwaite's method is based on an empirical correlation between mean air temperature and transpiration rates, and once the basic data have been evaluated requires only a knowledge of mean air temperature. It is relatively simple to compute, as it can be reduced to the reading of a nomogram, and can be used on a daily basis.

A knowledge of transpiration rates is important to irrigation agriculture for the determination of soil-moisture deficits. The only reliable way of measuring soil-moisture deficits is by the tedious routine of sampling, weighing, drying, and reweighing—the gravimetric method. Other methods such as the gypsum-block method (Bouyoucos and Mick 1940) suffer from difficulties of calibration and interpretation. However, if the deficit can be estimated reliably from readily available meteorological data, the timing of irrigation can be calculated within the range at which the moisture in the soil is sufficient to maintain transpiration at the potential rate. For these reasons an investigation was carried out to compare soil-moisture deficits as calculated by the

methods of Penman and Thornthwaite using as a standard the soil moisture as measured by the gravimetric method prior to each irrigation. The calculations were carried out over the irrigation periods of plots that were irrigated, by the border-dyke method, when the soil-moisture deficit in the top 12 inches was approximately 1.2 in., equivalent to 50% of available moisture. The assumption was made that, under these conditions, there was always sufficient moisture available to maintain transpiration at the potential rate. Results were available over four years of the trial. Thornthwaite's method had previously been applied to the first three of these irrigation seasons with a considerable degree of success (Rickard 1957). The irrigation periods of a second field experiment also irrigated by the border-dyke method, were used to evaluate Penman's factor. Both these areas were adjacent to the meteorological station.

The investigation was carried out on the Lismore stony silt loam on areas sown in permanent close-grazed sheep pasture of perennial ryegrass and white clover. The soil consists of about 7 in. of light-grey-brown stony silt loam over 7 in. of brownish-yellow stony silt loam. It is shallow, flat, well drained and mainly alluvial and aeolian.

Methods

Gravimetric Method

Details of the gravimetric method of determining the soil-moisture percentage and its conversion to the equivalent deficit are given by Rickard (1957).

Thornthwaite's Method

Details of this method, which was applied on a daily basis, are given by Mather (1950, 1954).

Penman's Method

Details of Penman's method are given in Pearl (1954) with the exception that the wind term was modified as suggested by Penman (1956) because of the results of the Lake Heffner Studies (U.S. Geological Survey 1952).

Estimation of Radiation Received

Since records of the radiation received were not available, the incoming radiation had to be calculated from the ratio of actual to possible sunshine hours and the theoretical radiation in the absence of an atmosphere. Investigations by Black *et al.* (1957) indicated a relationship of the type $R = R_A (a + b\ n/N)$ where R is the radiation actually received, R_A the theoretical radiation in the absence of an atmosphere and n/N the ratio of actual to possible sunshine hours. The records examined indicated that 'b' was approximately constant in the range 0.50 to 0.55 and the values of 'a' showed a marked trend being smaller at the higher latitudes than at the lower. Glover and McCulloch (1958a) were able to relate these regression coefficients to optical air mass, which varies with latitude, and in a further paper (1958b) showed that, in a range of stations ranging in latitude from 0° to 60°, b was approximately constant, being normally distributed

with mean 0.52 and S.E. 0.005. They found a highly significant regression ($P = 0.001$) of 'a' on latitude and showed that $a = 0.29 \cos \phi$. The derived relationship $R = R_A (0.29 \cos \phi + 0.52\ n/N)$ where ϕ is the latitude gave a good agreement with measured radiation and this was the form of the equation adopted for the investigation.

Determination of Penman's Factor

As Penman's method evaluates the evaporation from a hypothetical open water surface, Eo, use is made of a factor, f, to determine transpiration from pasture, E_T, by means of the equation, $E_T = f\ Eo$. Penman (1948) evaluated this factor for south-east England and pointed out in a discussion of its theoretical basis (Penman 1956) that the main determinant of f was hours of possible sunshine. The values of f for this locality were determined from the irrigation intervals of a second field experiment which was irrigated when the measured soil-moisture deficit was in the range 0.60 in. to 1.30 in. in the top 12 inches. The equation used was:

$$f = \frac{D + R}{\Sigma\ Eo}$$

where D is the measured deficit at the time of irrigation, R is the rainfall during the period, and Σ Eo is the total Penman evaporation for the period.

During some periods, rain was received in excess of the deficit; when this happened the equation was used over the shorter period, starting from the day after such rain fell. Because mean daily values of Eo for each month were used, it was apparent that the length of the period would have an effect on the derived value of f, and therefore weighted means were taken, the values of f being weighted by the length of the period. Table 1 shows the mean values for the five years studied, together with the values found by Penman to hold for south-east England.

TABLE 1. Values of Penman's Factor

Month	f	f England Corresponding Months
September	.6	.7
October	.6	.7
November	.6	.8
December	.7	.8
January	.7	.8
February	.8	.8
March	.7	.7

Results

Table 2 shows the calculated and measured deficits at each irrigation and Table 3 the seasonal totals for the four years of the investigation. The seasonal totals for Thornthwaite are within 2% of those determined by the gravimetric method and the seasonal totals for

TABLE 2. MEASURED AND CALCULATED DEFICITS AT IRRIGATION

Date and Period of Irrigation	No. of Days	Deficit at Irrigation (in.)			Difference from Measured Deficit (in.)	
		Measured	Penman	Thornthwaite	Penman	Thornthwaite
1953-54						
1. 1 Sep.–17 Nov.	77	1.44	1.39	1.67	—0.05	+0.23
2. 18 Nov.–1 Dec.	13	1.56	1.65	1.47	+0.09	—0.09
3. 2 Dec.–6 Jan.	36	1.34	1.43	1.16	+0.09	—0.18
4. 7 Jan.–18 Jan.	11	1.25	1.21	1.15	—0.04	—0.10
5. 19 Jan.–27 Jan.	8	1.12	1.30	1.16	+0.18	+0.04
6. 28 Jan.–19 Feb.	22	1.20	1.58	1.48	+0.38	+0.28
7. 20 Feb.–2 Mar.	10	1.14	1.37	1.23	+0.23	+0.09
Total		9.05	9.93	9.32		
Mean		1.29	1.42	1.33	+0.13	+0.04
1954-55						
1. 1 Sep.–5 Oct.	34	1.02	1.12	1.17	+0.10	+0.15
2. 6 Oct.–19 Oct.	13	1.20	1.18	0.77	—0.02	—0.43
3. 20 Oct.–10 Nov.	21	1.10	0.89	1.14	—0.21	+0.04
4. 11 Nov.–23 Nov.	12	1.15	1.30	1.24	+0.15	+0.09
5. 24 Nov.–4 Dec.	10	1.12	1.19	0.98	+0.07	—0.14
6. 5 Dec.–30 Dec.	25	1.24	1.14	1.44	—0.10	+0.20
7. 31 Dec.–6 Jan.	7	1.21	1.10	0.90	—0.11	—0.31
8. 7 Jan.–17 Jan.	10	1.24	1.22	1.25	—0.02	+0.01
9. 18 Jan.–1 Feb.	14	1.32	1.59	1.26	+0.27	—0.06
10. 2 Feb.–8 Feb.	6	0.98	0.90	0.87	—0.08	—0.11
11. 9 Feb.–25 Feb.	16	0.98	0.85	1.01	—0.13	+0.03
12. 26 Feb.–10 Mar.	12	1.01	0.99	1.37	—0.02	+0.36
13. 11 Mar.–4 Apr.	24	1.22	1.00	1.51	—0.22	+0.29
Total		14.79	14.47	14.91		
Mean		1.14	1.11	1.15	—0.03	+0.01
1955-56						
1. 1 Sep.–14 Oct.	43	1.25	0.88	1.08	—0.37	—0.17
2. 15 Oct.–2 Nov.	13	1.25	0.80	1.13	—0.45	—0.12
3. 3 Nov.–14 Nov.	11	1.10	1.03	0.84	—0.07	—0.26
4. 15 Nov.–7 Dec.	22	1.28	1.30	1.17	+0.02	—0.11
5. 8 Dec.–21 Dec.	13	1.22	1.52	1.60	+0.30	+0.38
6. 22 Dec.–5 Jan.	15	1.33	1.47	1.34	+0.14	+0.01
7. 6 Jan.–13 Jan.	7	1.44	1.44	1.08	0.00	—0.36
8. 14 Jan.–21 Jan.	7	1.18	1.46	1.39	+0.28	+0.21
9. 22 Jan.–10 Feb.	20	1.10	1.08	0.87	—0.02	—0.23
10. 11 Feb.–28 Feb.	17	1.31	1.58	1.47	+0.27	+0.16
11. 29 Feb.–12 Mar.	13	1.14	0.92	1.30	—0.22	+0.16
Total		13.60	13.48	13.27		
Mean		1.24	1.23	1.21	—0.01	—0.03
1956-57						
1. 1 Sep.–24 Sep.	24	1.31	1.10	1.11	—0.21	—0.20
2. 25 Sep.–26 Oct.	32	1.14	0.65	1.02	—0.49	—0.12
3. 27 Oct.–23 Nov.	28	1.18	1.22	1.27	+0.04	+0.09
4. 24 Nov.–31 Dec.	38	1.50	1.90	1.61	+0.40	+0.11
5. 1 Jan.–21 Jan.	21	1.25	1.47	1.17	+0.22	—0.08
6. 22 Jan.–1 Feb.	11	1.00	1.44	1.16	+0.44	+0.16
7. 2 Feb.–11 Feb.	10	1.31	1.43	1.25	+0.12	—0.06
Total		8.69	9.21	8.59		
Mean		1.24	1.32	1.23	+0.08	—0.01

Penman are within 10% of those determined by the gravimetric method. Penman (1950) regards values for annual totals within ± 10% as good from a consideration of the physical basis of the method.

TABLE 3. Seasonal Totals for Deficits (in.)

Season	Measured	Penman	Thornthwaite
1953/54	9.05	9.93	9.32
1954/55	14.79	14.47	14.91
1955/56	13.60	13.48	13.27
1956/57	8.69	9.21	8.59
Totals	46.13	47.09	46.09
Mean deficit at each irrigation	1.21	1.24	1.21

At the individual irrigations the 95% confidence limits for the deviations of calculated from measured deficit are ± 0.43 for Penman and ± 0.39 for Thornthwaite.

Discussion

If irrigation had been carried out using either of these methods of estimating the soil-moisture deficit it is likely that the use of irrigation water would not have been markedly different from that actually used. The agreement between the three methods is reasonably good and the following possible sources of error are apparent:

(a) The 95% confidence interval for the determination of soil-moisture percentage in the range under consideration is ± 1.3% (for the mean of six cores). This error may be further increased as the volume weight figure used to convert this percentage to inches of water per 12 in. depth of soil is an average figure for the area under consideration.

(b) Thornthwaite's method does not take into account the effects of wind and it would be expected that the effect of the strong Föhn winds experienced at Winchmore might be marked.

(c) Penman's method uses average figures which might mask periods of high transpiration.

A question of practical importance is the alteration in the timing of irrigations if irrigation had been on a calculated, instead of a measured, basis. To obtain an assessment of this the number of days earlier (—) or later (+) that the calculated deficit reached the measured deficit has been calculated for both methods. The results are given in Table 4.

One disadvantage of this type of comparison is that the daily transpiration rate is different at different times of the year. For example, in terms of total transpiration one day in December corresponds to four days in September. Deviations between calculated and actual

TABLE 4. Variations in Irrigation Dates Using Calculated Methods

No. of Days	Penman			Thornthwaite		
	Frequency of Difference		Cumulative Percentage	Frequency of Difference		Cumulative Percentage
0	9		24	4		11
	+	—		+	—	
1	6	8	61	8	11	61
2	—	5	74	7	4	89
3	2	3	87	—	2	95
4	2	—	92	1	—	97
5	—	—	—	—	—	—
6	2	—	97	1	—	100
7	—	—	—	—	—	—
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	1	—	100	—	—	—

dates of irrigation may therefore be greater in spring and autumn, but the importance of these deviations will be less. In general, the greatest deviations occur when daily transpiration is least.

The Penman method, however, suffers from a serious disadvantage if the radiation has to be estimated from sunshine hours. As the observers' readings have to be scaled they will not, in general, be available until some time after the period being studied has elapsed. A method utilising average values which are later corrected in the light of the weather conditions is outlined by Pearl (1954). An instrument for measuring net radiation directly has been devised by Suomi *et al.* (1954) which should enable Penman calculations to be made on a day-to-day basis if suitable computing equipment is available.

Conclusions

Since the methods of Thornthwaite and Penman give equally reliable results for the estimation of soil-moisture deficits as compared with those found by measurement by the gravimetric method, they have value, in this area, for estimating irrigation requirements. If irrigation had been carried out using either of these methods of estimating soil-moisture deficit, the use of irrigation water would not have been markedly different and there would have been only slight variation in irrigation dates. Since the Thornthwaite method is easier to use and available on a day-to-day basis it would seem that this is the method to be preferred in this area.

Acknowledgments

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EFFECT OF SHEARING TIME ON WOOL

VI. THE RATE OF GROWTH OF WOOL AND ITS RELATION TO TIME OF SHEARING

By L. F. Story and D. A. Ross, New Zealand Wool Industries Research Institute (Inc.), Dunedin

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Summary

Monthly measurements have been made of wool, grease, and suint production, and fibre diameter and length, in pre- and post-lamb-shorn Romney ewes over a period of 18 months. The growth of wool is some three times faster in summer than in late winter—early spring and the minimum rate decreases and occurs later with increasing number of lambs. Pre-lamb-shorn wool is sound because it is shorn near the time when fibre diameter is at a minimum, and wool shorn at any other time has a thin region where it is likely to break.

Introduction

There has been considerable prejudice against pre-lamb-shorn wool, partly because this was a new and unfamiliar type of wool, differing in appearance and yield from established types, and partly because some lots of wool offered as pre-lamb-shorn were much shorter than the usual clips. It has also been said that it is unnatural to shear wool in the winter as it is not mature and the "yolk has not risen".

Many clips have been short because they had had only 9 months' growth due to changeover in shearing time from after to before lambing, and so they were not true pre-lamb-shorn wools. However, farmers and woolstore staffs have stated that full 12 months' wools are shorter and lighter than post-lamb-shorn wools. Such statements are based on a comparison of clips with what were produced on the same properties two or more years before, or with clips from different flocks in other localities. In both cases it is very difficult to make a comparison when the amount of wool grown can be affected so much by other factors such as weather and feed conditions in the one case and differences between flocks and in management in the other case. A true comparison can only be made by shearing parts of a single flock of sheep at the two different times, and, as reported in Part IV of this series (Story and Ross 1959), this has been done and no consistent differences in fleece weight or length were found, but the pre-lamb-shorn wool was vastly superior in its freedom from wool faults.

At the same time as the flock trials, measurements have been made of the rate of growth of wool on some of the crossbred ewes, firstly with quarterly sampling, and secondly a more detailed study with monthly sampling. The monthly production of grease and suint was also observed in the second trial.

These experiments have been made to find just what relationship there is between the shearing times and the rate of growth of wool,

why pre-lamb-shorn wool is so relatively free from faults, and whether grease and suint do in fact noticeably increase in quantity in the late spring.

Experimental

All these experiments were conducted on Romney crossbred ewes at the Department of Agriculture's Invermay Research Station, near Mosgiel.

Quarterly Sampling, 1954

Eight sheep were selected at random from each of the pre-lamb-shorn and post-lamb-shorn sections of the Romney flock, being shorn in August and November, 1953, respectively. Samples of wool were clipped from the same (unmeasured) patch on the midside of the sheep, using a small shearing handpiece, in March, June, August, and November, 1954. The first pre-lamb sample had thus had 6 months' growth, while the rest of the samples had had approximately 3 months' growth each.

The lengths of 5 staples per sample were measured after straightening against a ruler, and fibre-length measurements were made using a WIRA single fibre length machine.

Quarterly Sampling, 1954-5

As a further refinement to eliminate all differences other than the time of shearing, 4 sheep were shorn on one side only in August 1954, the other side not being shorn until November. Samples were taken as before, but from both midsides, in November 1954, and March, June, August, and December, 1955, and measurements of staple length and single-fibre length were made on each sample.

Monthly Sampling, 1954-6

A more detailed study of the rate of growth of wool was made using 10 full-mouth Romney ewes. Five of them were pre-lamb shorn on 3 September 1954 and 5 September 1955, and the other 5 were post-lamb-shorn on 27 November 1954 and 2 December 1955. The sheep were kept on grass or grass/lucerne pastures throughout the trial, with supplements of swedes in the winter.

On 3 September 1954 an area on the midside, approximately 10 cm square, was shorn with a small handpiece and its edges trimmed straight with angled scissors. The same patch was re-shorn at the beginning of each month up to March 1956. At shearing time a band about 2 in. wide was first shorn around the sampling area leaving the wool about an inch long, and the rest of the fleece was then shorn as usual. Thus the boundaries of the sampled area were clearly defined as the junction of short wool with longer wool. The area of skin being sampled was measured by callipers while held taut by two assistants.

Weight and Analysis of Samples. The monthly greasy samples were exposed in the conditioning-room at 68°F and 65% r.h. and weighed. A portion of about 2 g was then weighed off and packed into a WIRA rapid-extraction tube (Dickinson and Palmer 1951). The

grease was extracted by petroleum ether, the solution collected in a small flask, the solvent evaporated and the residue weighed. Solvent remaining in the wool was evaporated by drawing warm air through the tube and the suint was next extracted by warm distilled water, the solution being collected in an aluminium dish and evaporated on a hot plate, and the residue quickly weighed. The wool was then pulled out of the tube, rinsed in a beaker of warm water to remove any dirt, dried in the oven at 105°c, and transferred to a weighing bottle for determining the clean dry weight. From the results the weights of clean dry wool, grease, and suint produced per day per 100 sq cm of skin in each period were calculated. (Each period was approximately a calendar month.)

Fibre Diameter and Length. The fibre diameter was measured by the projection microscope on cross sections taken at the centre of each sample, 500 measurements being made per sample. The fibre length was measured by straightening fibres alongside a millimetre scale using two pairs of tweezers, tufts containing 150–200 fibres being measured. The mean fibre lengths were divided by the appropriate number of days to obtain the mean daily rate of growth in each period.

RESULTS

Quarterly Sampling

As there were only 8 sheep in each group in the first experiment, a comparison of the actual lengths in each period between the two groups is of little significance because of normal variations between sheep. More significant figures are the percentages of the 12 months' total that were grown in each of the periods, and these were found to be little different as shown by the fibre length data given in Table 1. Thus the different shearing-times had no noticeable effect in this experiment.

TABLE 1. FIBRE LENGTHS OF QUARTERLY SAMPLES

Date of Sampling 1954	Period of Growth Weeks	Pre-lamb-shorn		Post-lamb-shorn	
		Length cm	% of total	Length cm	% of total
1 Mar.	27.0	10.1			
	(13.5	(5.9*	32.2)	5.9	32.4
3 June	13.5	5.3	29.0	5.0	27.5
13 Aug.	10.0	2.6	14.2	2.8	15.4
23 Nov.	14.5	4.5	24.6	4.5	24.7
Total	51.5	18.3	100.0	18.2	100.0

* Estimated from the 6 months' length and the 3 months August to November, 1954.

Measurements of staple length gave almost identical results as percentages, as would be expected. However, it was observed that whereas the totals of the four mean fibre lengths were in reasonable agreement with the mean fibre lengths of 12 months' staples taken

alongside the clipped area, there was a considerable discrepancy with staple lengths as the 12 months' staples were noticeably shorter than the sum of the four 3-monthly sections taken separately.

The results on the "half-shorn" sheep were very similar, the totals of the mean fibre lengths for the two sides of the four sheep over the 5 sampling periods differing by only one per cent. Thus, while the different shearing times for the two sides of the sheep caused a large difference in the style grading and strength of the wool (Story and Ross 1959), there was little effect on the length.

Monthly Sampling

The results which follow are all given, where applicable, as growth or production per day per 100 sq cm of skin. "Month" always refers to the calendar month in which the sample was mainly growing, though the samples were actually clipped about the 3rd of the following month.

Weight of Wool

There was a very large variation in wool production between sheep and between months. As there were only 5 sheep in each group, no significance can be attached to the relative amounts of wool produced by the two groups in any particular month. As it happened, most of the means for the pre-lamb-shorn group were considerably higher than for the post-lamb-shorn group, but this was mainly because the former

TABLE 2. Daily Production of Clean Dry Wool

Month (1954-6)	Weight mg/day/100 cm²			Percentage	
	A*	B*	All	A	B
Sep.	18	43	30	1.8	5.6
Oct.	60	58	59	6.1	7.6
Nov.	64	54	59	6.5	7.0
Dec.	107	85	96	10.9	11.1
Jan.	120	95	108	12.2	12.4
Feb.	123	101	112	12.5	13.1
Mar.	89	87	88	9.1	11.3
Apr.	88	82	85	9.0	10.7
May	100	75	88	10.2	9.8
Jun.	84	55	69	8.5	7.2
Jul.	43	34	38	4.4	4.4
Aug.	35	26	31	3.6	3.4
Sep.	39	25	33	4.0	3.3
Oct.	63	44	54	6.4	5.7
Nov.	90	58	75	9.2	7.6
Dec.	110	77	95	11.2	10.0
Jan.	88	85	87	9.0	11.1
Feb.	89	80	85	9.1	10.4

* A = pre-lamb shorn; B = post-lamb shorn. Horizontal bars indicate shearing times.

group included one sheep that was a very high producer for most of the year, while the latter group included one sheep that was a very low producer. To enable a comparison to be made between the two groups, the daily productions in each month have been calculated as percentages of their total for the 12 months' period December 1954 to November 1955.

The figures show no consistent differences in wool production between the two groups. Thus while the percentage production of the pre-lamb-shorn sheep was the lower for the 3-months period following shearing in 1954, the position was reversed for the same period in 1955. Lambing data given below show that these variations were primarily due to different numbers of lambs rather than to any effect of shearing time.

The mean weights of wool per day in each month for all the sheep have been plotted in Fig. 1 which shows the marked seasonal rhythm in wool growth similar to that reported by other workers (Coop 1953; Hart 1955).

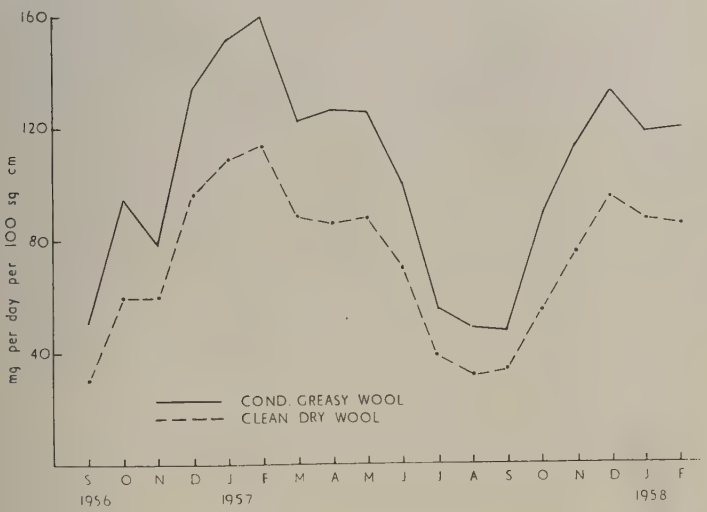

Fig. 1.—Seasonal variation in rate of wool growth.

In the present case the mean variation in wool production per day showed a range of 3.3 to 1 for conditioned greasy wool and 3.6 to 1 for oven-dry clean wool, while the maximum range for one of the sheep was almost 10 to 1.

Effect of Pregnancy and Lactation

It was observed that pregnancy and lactation had a noticeable effect on wool growth in the winter months, causing the minimum wool production in the winter to be rather lower and later than for dry sheep.

As only 10 sheep were being studied, the data available are very limited. However they are sufficiently consistent with one another to give a fair indication of the probable course of events. The individual figures for clean dry wool production for each sheep over the 1955 lambing period (lambs were dropped in September) are given in Table 3.

TABLE 3. EFFECT OF LAMBING ON WOOL PRODUCTION

No. of lambs	Sheep No.	Shorn	mg/day/100 cm²				min./max. %†
			Jul	Aug	Sep	Oct	
0	1876	Pre	50	48	75	77	41
0	1919	Post	27	26	36	42	40
1	1917	Pre	46	40	46	65	39
1	1965	Pre	38	33	34	48	26
1	1863	Post	39	32	20	57	20
2	244	Pre	41	27	19	73	10
2	1892	Pre	39	29	22	50	21
2	256	Post	30	21	18	43	16
2	1951	Post	40	29	26	34	26
?	391*	Post	32	23	—	—	—

* Died in September before lambing; its low August wool production may have been due to illness.
† Minimum production as percentage of maximum during the year.

The following observations can be made on these results:

i. In dry sheep, the minimum wool growth occurred in July/August.
ii. In sheep with one lamb the minimum occurred in August/September.
iii. In sheep with twin lambs the minimum occurred in September.
iv. Wool growth decreased more compared with the maximum during the year as the number of lambs increased.

In 1954, four of the pre-lamb-shorn sheep had twin lambs with a corresponding very low wool production in September, while the fifth had a single lamb with an equally low wool production. On the other hand the post-lamb-shorn sheep had one or no lambs in 1954 and in consequence their wool production was not depressed to the same extent. This is the probable explanation of the large difference in wool production between the two groups in September 1954.

These results, limited as they are, thus do indicate that lambing, especially when there are twin lambs, does have a marked depressing effect on wool growth. At no time were the ewes short of feed.

Fibre Length and Diameter

There was little difference in fibre diameter and length grown per day between the means for the two groups of sheep in successive months, so overall means only will be quoted. The mean fibre length in mm/day ranged from 0.39 to 0.66, while the mean diameter ranged from 28.5

to 41.9 microns. The variation in fibre length and diameter from month to month is shown by the results given in Table 4, in which the coefficients of variation are also included. It will be noted that the c. of v. apparently increases when the length or diameter decreases, and this is taken to mean that, when fibre growth slackens in the winter, some of the follicles are affected more than others, so that the percentage variation between fibres increases.

TABLE 4. FIBRE LENGTHS AND DIAMETERS

Month	Length		Diameter	
	Mean mm/day	C.V. %	Mean microns	C.V. %
Sep 1954	0.39	18.8	32.1	24.6
Oct	0.52	16.0	36.9	22.4
Nov	0.61	16.1	37.3	21.2
Dec	0.66	13.0	40.5	20.4
Jan 1955	0.64	14.4	41.5	19.5
Feb	0.63	14.4	41.9	19.1
Mar	0.59	12.8	40.0	19.1
Apr	0.59	15.2	38.7	20.5
May	0.56	15.9	38.6	19.5
Jun	0.49	17.7	35.3	20.9
Jul	0.39	18.7	31.9	22.0
Aug	0.41	18.7	28.5	23.2
Sep	0.43	18.5	30.2	23.8
Oct	0.48	18.6	34.1	21.9
Nov	0.53	17.8	36.9	19.7
Dec	0.56	19.3	39.9	19.8
Jan 1956	0.51	18.4	39.2	19.7
Feb	0.55	14.9	39.8	19.9

It may also be noted that the maximum and minimum growth in fibre length per day occur about one month before the corresponding maximum and minimum fibre diameters, which indicates that the mechanisms controlling fibre length and diameter are at least partly independent of one another.

As with the quarterly sample results mentioned earlier, the total of the monthly sample staple lengths for the full year was considerably longer than the staple length of the full-year fleece samples. The mean daily rates of growth calculated from the 3 sets of data for a full year's growth were as follows:

Calculated from	Pre-lamb-shorn mm/day	Post-lamb-shorn mm/day
Full-year staple length	0.45	0.38
Total of monthly sample staple lengths	0.52	0.46
Total of monthly sample mean fibre lengths	0.56	0.51

For all the ewes, the total of the monthly staple lengths was 1.19 times the full year sample staple length, while the fibre length of the monthly samples for the same period was 1.08 times the staple length.

It is possible that the more frequent the sampling, the greater the difference between the total of the sample staple lengths and the length of the uncut staple grown over the same period, as each of the monthly samples had a fairly straight tip region, and well defined crimp was not formed in this short period. This would also explain why the total of the four 3-monthly staple lengths was considerably higher than the full year's staple length because four tip regions would be involved in this case, while the totals of the four 3-monthly fibre lengths agreed with the fibre length of the full year samples.

Grease and Suint Production

Wool grease and water-soluble suint are secreted from separate glands associated with the wool follicles, and the activity of the glands varies very considerably from sheep to sheep, and also from month to month. Since the sheep in this trial were kept in a paddock without shelter, they were exposed to rain, which had a tendency to leach out the suint and to a lesser extent grease, especially in the first 2 or 3 months after shearing while the wool around the sampling area was too short to offer any protection. Thus the figures obtained for suint production were very erratic and in several cases were undoubtedly considerably lower than the actual production.

As there was little difference in mean percentage of grease between the two groups of sheep in most months, and no apparent shearing effect, the data for the whole 10 sheep were combined. There were on the other hand very large differences in mean suint content of the two

TABLE 5. Grease and Suint Production

Month (1954-6)	Grease		Suint			
			%*		mg/day/100 cm²	
	%*	mg/day/100 cm²	A†	B†	A	B
Sep	14.2	3.7	14.6	26.4	2.5	11.4
Oct	14.3	8.2	11.9	25.7	7.2	14.8
Nov	7.6	4.5	7.3	4.2	4.4	2.3
Dec	10.2	9.8	8.0	7.7	8.1	6.6
Jan	8.0	8.5	10.0	9.8	12.0	9.4
Feb	9.3	10.4	11.8	11.4	14.1	11.8
Mar	10.3	9.1	6.4	6.7	5.5	5.9
Apr	10.0	8.5	14.9	13.5	13.5	11.3
May	11.0	9.4	11.2	12.8	11.1	9.7
Jun	13.0	8.4	10.0	11.0	8.0	6.2
Jul	14.6	5.6	7.8	8.8	3.5	3.0
Aug	13.2	4.0	18.8	14.5	6.6	4.1
Sep	20.2	6.4	11.4	22.3	4.4	5.8
Oct	12.6	6.7	6.7	28.0	4.1	13.0
Nov	10.8	8.0	10.9	24.4	9.7	14.4
Dec	9.8	9.5	12.3	7.2	13.2	5.6
Jan	11.7	9.2	10.9	5.3	9.3	4.1
Feb	12.6	10.1	6.1	5.6	5.4	4.5

* On clean oven-dry weight of wool.
† A = pre-lamb-shorn group; B = post-lamb-shorn group.

groups around the shearing times, so separate results are given as a basis for discussion. The results of grease and suint analyses are given in Table 5 in two forms, viz, percentages on the clean dry weight of wool grown in the same period, and milligrammes of grease or suint produced per day per 100 sq cm.

Grease. The percentage of grease was high in winter and early spring, the highest figure being recorded in Sep. 1955, while the lowest figures were recorded in the summers, January-February and December 1955. (The low figure for November 1954 was exceptional, as it was due to low results for the post-lamb-shorn group which had been shorn 6 days before the sample was taken thus exposing the sampling area to rain.)

Examination of the grease-production figures discloses however that the trend in actual production followed the trend for wool-fibre growth, the grease production being highest in summer and lowest in winter. The variation in mean grease production was however less than in wool production which explains why the percentage of grease increased in the winter.

Suint. The suint results, though somewhat erratic because of unknown losses caused by weathering, are most interesting. Considering first the percentage figures for the post-lamb-shorn sheep, it will be seen that these reach a very high level in the spring months just before shearing, and after shearing there is an abrupt fall in the percentage of suint. This fall could be due to the effect of shearing, but is more likely simply due to exposure of the newly grown wool to rain. Thus in October 1954 the percentage of suint was 25.7, but in November it was only 4.2. Yet the November sample was taken only 6 days after the fleece was shorn, i.e. the wool had been growing under the same conditions as for the October sample for most of the period. On the other hand the November 1955 sample was taken on the same day that the fleece was shorn and it contained 24.4% suint, while the next (December) sample contained only 7.2%.

The pre-lamb-shorn results do not reach such high levels, probably because shearing exposes the newly growing wool to rain, though it is also possible that the effect of shearing could be to decrease the secretion of suint.

The suint production figures are also instructive. They show that the actual amount produced was apparently high in February-April (late summer-autumn), low in July-August (winter), and high again in October-November in the sheep that had not been shorn. They thus also show a seasonal rhythm, with an additional high level during the period of lactation in the unshorn sheep. It is possible that this high level in the late spring is actually part of the seasonal cycle, the increase commencing earlier for suint than for grease and wool production, but being interrupted by shearing.

Dipping is another factor in the amount of suint remaining on the wool. This is the probable cause of the low figures recorded for suint in March 1955, though it is known too that the sheep were not doing well in that month as there was an average loss in body weight of 14 lb

in March followed by a gain of 21 lb in April, and also the wool production in March was rather less than might have been expected.

General Discussion

This work was initiated with a view to obtaining further information on the effect of shearing before lambing on the rate of growth of wool, as compared with shearing after lambing. Previous papers (Story and Ross 1959; Story 1959) have shown that there is apparently no consistent difference in the weight and length of the twelve-month fleeces, though there is a large difference in style grading in favour of the pre-lamb-shorn wool. However the latter wool was not liked in the wool trade in the early years of pre-lamb shearing, and various remarks were passed in the trade to the effect that it was not "mature" or "fully-grown ready for shearing", with which was associated the further statement that "the yolk had not risen".

Our observations are in agreement with those of other workers in showing that the rate of growth of wool is much higher in the summer than in the winter, the average ratio between maximum and minimum rates being 3.6 to 1 for the 10 ewes studied. The minimum rate of growth occurred in July-August for dry ewes, and about a month later for wet ewes, the relative decrease in wool growth also being noticeably greater for the latter.

It is in the winter to early spring period that fibre shedding is known to occur, this being much more pronounced in the coats of primitive sheep. In fibre shedding, of course, the rate of growth of the shed fibres has decreased to zero for a time. The free fibre root ends thus formed become felted and tangled together producing the familiar cotted fleece. The slackening of wool growth in the winter and fibre shedding and the associated cotting are thus related phenomena.

Wool fibres are normally growing continuously but at a varying rate, and this is illustrated diagrammatically in Fig. 2 in which the data previously given relating to the fibre diameter and the length grown per day in each month are plotted at right angles to one another, the diameter being much magnified in relation to length. It will readily be seen that pre-lamb shearing in winter results in fibres that taper at both ends, while shearing in spring or summer after lambing results in fibres that are thick at the end and have a thin region towards the centre.

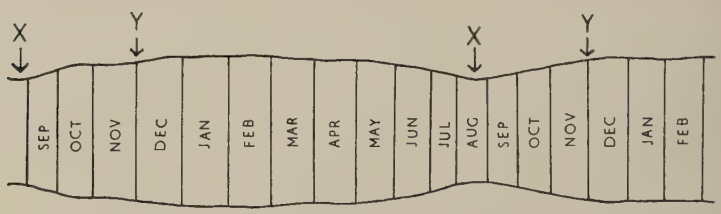

Fig. 2.—Diagram showing relation of shearing times to fibre shape. X = pre-lamb shearing time; Y = post-lamb shearing time.

Hence the pre-lamb-shorn wool will be sound while that shorn at a later time will have a weak region some distance up from the butt. It would thus appear that the optimum time to shear sheep is in the winter when wool growth is near its minimum, thus avoiding tenderness and cotting.

Thus the different features of pre- and post-lamb-shorn wool, especially the greater strength and freedom from faults of the former, can be logically explained on the basis of changes in dimensions of the wool fibre during the year. Further there is no reason why the quantity of clean wool grown in a 12-month period should be affected by different times of shearing, except insofar as the shearing itself may have different direct or indirect stimulating effects on the rate of growth at different times, depending to a large extent on the feed supply available. Any such stimulation is likely to occur for only a limited period, and no consistent differences were observed between groups when wool samples were taken at monthly intervals.

"Rise of Yolk". It is often noticed when shearing in November that the base of the fleece contains a large amount of suint and grease which may be semi-fluid making the wool very sticky, or sometimes may be dry and caked. This secretion is often yellow in colour (scourable) or may be accompanied by canary staining of the wool (unscourable). Wool shorn before lambing rarely shows any deep-yellow colouration or caky yoke.

Examination of the data on the amounts of grease and suint produced per month shows that the popular explanation in terms of increased "yolk" production at this time is to a large extent fallacious. The increased percentages of grease and suint are actually mostly due to the much diminished rate of wool-fibre production. Grease production also diminishes in the winter though to a much less extent than the wool, while suint production shows a similar tendency, though an early rise in suint production was observed in some of the unshorn sheep during the period of lactation. As suint is very hygroscopic, this resulted in the wool samples taken at that time being very moist and sticky owing to the high percentage of suint. Samples from pre-lamb-shorn sheep at that time contained much less suint, either from loss by weathering or lower suint production after shearing. Further investigations of suint production need to be carried out with the sheep under shelter, e.g. rugged or stall fed, though such artificial conditions may themselves affect the rate of production of suint, grease, and wool.

Temperature may be another factor in this connection, the rising spring temperatures rendering the wool grease more fluid and therefore more noticeable.

Yield. The clean yield of pre-lamb-shorn wool is usually found to be higher than that of post-lamb-shorn. Two of the probable factors causing this are firstly that the wool growing in the period September-November has a lower suint content in the case of the pre-lamb-shorn, and secondly at the time of dipping in February-March, the pre-lamb-shorn sheep are carrying 3 months' more wool which is exposed to leaching during dipping.

Conclusions

1. The rate of wool growth in Romney crossbred ewes varies considerably during the year, being highest in the summer and lowest in the late winter-early spring period. Pregnant ewes show a greater reduction in wool growth than non-pregnant ewes and the minimum occurs a few weeks later.

2. Both the length grown per day and the diameter vary in an annual cycle, but the maximum and minimum in length occur some weeks earlier than for diameter.

3. The optimum time for shearing sheep to obtain sound wool is in the winter-early spring period when the mean fibre diameter is at a minimum.

4. Shearing at this time results in very sound wool free from faults such as tenderness, cotting and yellow discolouration.

5. The increase in percentage of grease and suint in early spring is largely due to the low rate of growth of wool fibre at this time rather than to an increased production of grease and suint, though the latter does tend to increase rather rapidly during the period of lactation.

Acknowledgments

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THE EFFECTS OF FREQUENT HARD GRAZINGS AT DIFFERENT TIMES OF THE YEAR ON THE PRODUCTIVITY AND SPECIES YIELDS OF A GRASS-CLOVER PASTURE

By R. W. Brougham, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

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Summary

An experiment is described in which the effects of frequent hard grazings at different times of the year on the productivity and species yields of a pasture of short-rotation ryegrass, cocksfoot, and red and white clover were determined over an eighteen-month period.

Frequent hard grazings during the winter encouraged the growth of all sown species, and dry-matter (d.m.) yields were high for these pastures after a change to less-intensive grazing in later seasons.

Frequent hard grazings during the spring reduced immediate d.m. yields markedly but because there were no detrimental effects on plant survival of any of the sown species except red clover, subsequent d.m. yields of these pastures were also relatively high when the management system reverted to the less-intensive system.

Frequent hard grazings during the summer resulted in widespread death of all sown species except white clover. Subsequent d.m. yields obtained from these pastures were thus severely reduced.

Frequent hard grazings during the autumn reduced immediate d.m. yields by approximately 20% but, it is suggested that because the light-shielding clover canopy was controlled, yields obtained from the sown grass species in the following seasons were high.

The results highlight the importance of summer grazing management to ensure persistency of pasture species, the importance of intensive grazing in the late autumn to bring about a rapid change in botanical composition from dominance of summer-growing species to dominance of winter-growing species, and the relatively high yields obtained by avoiding long spelling during the winter. The results are discussed in relation to existing pasture-management principles and productivity.

Introduction

In New Zealand, herbage production normally exceeds animal requirements during the spring and autumn, but, depending on climate and locality, growth from pastures during the summer and winter may not be sufficient for the daily feed requirements of the animals (McMeekan 1945). For this reason pastures are often subjected to overgrazing during the summer and winter. Under adverse climatic conditions overgrazing may also occur during the spring and autumn.

Where rotational grazing is practised, overgrazing is associated with a decreasing spelling interval between grazings and a much more intensive defoliation of herbage at each grazing. The results obtained in a previous study (Brougham 1959b) show that this pasture management leads to low herbage yields and undesirable changes in botanical

composition. Where grazing is severe during the summer, widespread death of desirable pasture plants occurs. This naturally has a marked effect on subsequent production.

For this reason it was considered desirable to obtain data on the effects of hard grazings for different periods of the year on immediate and subsequent productivity of rotationally grazed pastures. The experiment reported here was designed to determine the effects of these factors on a pasture of short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*), cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*), red clover (*Trifolium pratense*), and white clover (*T. repens*).

Experimental

The experiment was carried out at the Palmerston North station of the Grasslands Division on Manawatu silt loam. Before ploughing in March 1955, the area had been in pasture for a period of two years.

In late March 1955, the area was sown with short-rotation ryegrass at 20 lb, cocksfoot at 5 lb, timothy at 3 lb, red clover at 4 lb, and white clover at 3 lb, per acre. All seed was of pedigree origin. Superphosphate at 2 cwt per acre was applied at sowing, together with 8 lb per acre of 50% equivalent DDT for grass-grub control. Further annual dressings of superphosphate at 2 cwt per acre were applied each autumn.

After an establishment period of twelve months and a further three-month period of frequent but lax grazings, the following six treatments were applied as from 1 June 1956:

(1) Frequent hard grazings from 3 in. to 1 in. throughout the year (3–1).

(2) Frequent hard grazings from 3 in. to 1 in. during the winter with frequent less intensive grazings from 7 in. to 3 in. for the remainder of the year (3–1 winter).

(3) Frequent hard grazings from 3 in. to 1 in. during the spring with frequent less intensive grazings from 7 in. to 3 in. for the remainder of the year (3–1 spring).

(4) Frequent hard grazings from 3 in. to 1 in. during the summer with frequent less intensive grazings from 7 in. to 3 in. for the remainder of the year (3–1 summer).

(5) Frequent hard grazings from 3 in. to 1 in. during the autumn with frequent less intensive grazings from 7 in. to 3 in. for the remainder of the year (3–1 autumn).

(6) Frequent less intensive grazings from 7 in. to 3 in. throughout the year (7–3).

For the purpose of the experiment, June, July, and August were defined as winter; September, October, and November as spring; December, January, and February as summer; and March, April, and May as autumn.

Treatments 1 and 2 were grazed to 1 in. on the starting date and the remainder of the treatments to 3 in. The treatments as described above were then adhered to except that the last grazing of each treatment in each season was made to coincide with the end of the particular season regardless of the height of herbage before grazing. Also for

treatments 3, 4, and 5, at the changeover of each from the 7–3 to the 3-1 systems whenever it occurred, the herbage was first grazed to 3 in. and the yield measured. Immediately after it was grazed to 1 in. and a stubble yield recorded.

At the beginning of the winter in 1957 all treatments were subjected to the 7–3 system of management for a period of approximately 6 months.

The six treatments were replicated three times in randomised blocks. The area of each plot was approximately 1/15 acre and each was fenced so that grazings with sheep could be carried out separately. The grazing procedure used was the same as that described in a previous experiment (Brougham 1959b) as was the method used for herbage yield determinations and botanical separations.

The experiment ended on 21 November 1957.

RESULTS

Total Dry-matter Production and Species Yields

Yields are expressed throughout in lb d.m. per acre. Because of the small amounts of d.m. obtained from timothy throughout the experiment, they have been included in the yields obtained from "Other Grasses". Because the resulting yields of "Other Grasses" and those of "Other Species" were small fractions of the total yield, statistical analyses are not presented.

Seasonal Yields

Winter 1956. Table 1 shows total and species yields for the six treatments. The least significant differences at the 5% level are also shown.

TABLE 1. WINTER YIELDS (1956)
(lb/ac)

Treatment	Total Yield	Species Yields					
		Ryegrass	Cocksfoot	Other Grasses	Red Clover	White Clover	Other Species
1 (3–1)	1850	1430	125	50	100	130	15
2 (3–1 Winter)	1760	1430	105	35	95	90	5
3 (3–1 Spring)	1010	880	55	5	40	30	—
4 (3–1 Summer)	910	770	60	10	40	30	—
5 (3–1 Autumn)	1080	960	50	20	30	20	—
6 (7–3)	1060	940	80	10	20	10	—
Sig. diff. at 5% level	400	403	42	—	28	39	—

The treatments below the dotted line are those that had received the same treatment to the end of the winter.

The following features are shown:

(i) Frequent hard grazings throughout the winter (treatments 1 and 2) yielded more than the laxly grazed treatments (3, 4, 5, and 6).

(ii) The increases in yield in treatments 1 and 2 were due to significant increases in the yields of all sown and unsown species.

Spring 1956. Total and species yields obtained from the six treatments during the spring of 1956 are shown in Table 2. Also shown are the least significant differences at the 5 % level.

TABLE 2. SPRING YIELDS (1956)
(lb/ac)

Treatment	Total Yield	Species Yields					
		Ryegrass	Cocksfoot	Other Grasses	Red Clover	White Clover	Other Species
1 (3–1)	3420	2570	285	165	100	245	55
2 (3–1 Winter)	4750	3300	375	75	585	405	10
3 (3–1 Spring)	3100	2100	445	155	175	205	20
4 (3–1 Summer)	4870	3000	610	155	745	330	30
5 (3–1 Autumn)	4810	3270	930	240	205	165	—
6 (7–3)	4520	3240	550	120	380	225	5
Sig. diff. at 5% level	489	316	225	—	257	112	—

The treatments below the dotted line are those that had received the same treatment to the end of the spring.

The following are the main features:

(i) Total yields obtained from the pastures grazed hard throughout the spring (treatments 1 and 3) did not differ significantly and no differences were recorded in the yields of all sown or unsown species except for ryegrass which showed a small difference in favour of treatment 1 over treatment 3.

(ii) Total yields obtained from treatments 1 and 3 were lower than those obtained from the pastures grazed less intensively during the spring (treatments 2, 4, 5, and 6). These differences were due to significant decreases in ryegrass and clover, particularly red clover.

(iii) The comparison between the treatment grazed hard during the preceding winter but less intensively during the spring (2) and the other laxly grazed pastures during the spring (treatments 4, 5, and 6) showed no significant differences between total yields or those obtained from ryegrass and other unsown species or grasses. However, yields of cocksfoot from treatment 2 were less than those obtained from the other treatments but this decrease was offset by increased yields of both red and white clover.

(iv) There were no significant differences between the total yields obtained from treatments 4, 5, and 6, although there were some small botanical differences. Treatment 5 contained more cocksfoot than the other two treatments in the comparison and treatment 4 contained more red and white clover.

Summer 1956–57. Table 3 shows total and species yields obtained from the six treatments during the summer, and the least significant differences at the 5% level.

TABLE 3. SUMMER YIELDS (1956-57)
(lb/ac)

Treatment	Total Yield	Species Yields					
		Ryegrass	Cocksfoot	Other Grasses	Red Clover	White Clover	Other Species
1 (3–1)	2420	1370	195	95	25	520	215
2(3–1 Winter)	4560	1990	675	40	1060	700	95
3 (3–1 Spring)	3580	1740	650	75	365	575	175
4 (3–1 Summer)	1990	1390	265	55	65	185	30
5 (3–1 Autumn)	3680	1330	1050	135	735	305	125
6 (7–3)	3960	1480	1000	105	745	545	85
Sig. diff. at 5% level	761	506	302	—	630	216	—

The treatments below the dotted line are those that had received the same treatment until the end of the summer.

The following are the main features of Table 3:

(i) Total yields obtained from the pastures grazed hard throughout the summer (treatments 1 and 4) did not differ significantly. However, there were some small botanical differences. A higher yield was obtained from white clover and from "other species" in treatment 1.

(ii) Lower total yields were obtained from treatments 1 and 4 than from the pastures grazed less intensively during the summer (treatments 2, 3, 5, and 6). These decreases were due mainly to marked reductions in the yields of cocksfoot and red clover. Yields obtained from the other sown species were similar except that the yield of white clover in treatment 4 was less than the yields obtained from the other treatments in this comparison.

(iii) The comparison between the treatment hard grazed in winter (2) and the less intensively grazed treatments (5 and 6), showed that a higher yield was obtained from treatment 2. This was due to increased yields of ryegrass and white clover although cocksfoot yielded less.

(iv) No significant differences in total yield were obtained between the treatment grazed hard in the spring (3) and treatments 5 and 6.

However, cocksfoot and red clover did yield significantly less in treatment 3 but this was offset by a non-significant increase in the yield of ryegrass.

(v) The total yield obtained from treatment 2 was higher than that obtained from treatment 3 because of a marked increase in red-clover yield. Yields obtained from other sown species did not differ significantly.

(vi) The less intensively grazed treatments (5 and 6) did not show any marked differences in total or species yields.

Autumn 1957. Table 4 shows total and species yields obtained from the six treatments during the autumn, and the least significant differences at the 5% level.

TABLE 4. Autumn Yields (1957)
(lb/ac)

Treatment	Total Yield	Species Yields					
		Ryegrass	Cocksfoot	Other Grasses	Red Clover	White Clover	Other Species
1 (3–1)	1650	115	20	20	20	1220	255
2 (3–1 Winter)	2280	365	985	20	405	475	30
3 (3–1 Spring)	1910	185	1100	20	205	375	25
4 (3–1 Summer)	970	125	55	10	50	695	35
5 (3–1 Autumn)	1810	220	1195	25	110	225	35
6 (7–3)	2160	120	1240	25	440	325	10
Sig. diff. at 5% level	224	146	370	—	228	234	—

The features of Table 4 are:

(i) Total yields obtained from the pastures grazed hard throughout the autumn (treatments 1 and 5) did not differ significantly, but there were marked botanical differences. The dominant species of treatment 1 was white clover whereas that of treatment 5 was cocksfoot. Yields obtained from unsown species were also relatively high in treatment 1. Other differences did not reach significance.

(ii) Total yields from the pastures that were hard grazed in the summer (treatment 4) were much lower than those from the other five treatments. White clover was also dominant in this treatment, as in treatment 1.

(iii) In the other less intensively grazed pastures (treatments 2, 3, and 6), treatment 3 yielded less total d.m. than either 2 or 6. This was due mainly to a reduced yield of red clover.

Annual Yields

Total and species yields of the six treatments for the full year of the experiment are shown in Table 5, and also the least significant differences at the 5% level.

TABLE 5. ANNUAL YIELDS
(lb/ac)

Treatment	Total Yield	Species Yields					
		Ryegrass	Cocksfoot	Other Grasses	Red Clover	White Clover	Other Species
1 (3–1)	9340	5480	630	325	250	2115	540
2 (3–1 Winter)	13360	7080	2140	170	2150	1680	140
3 (3–1 Spring)	9600*	4900	2260	255	785	1180	220
4 (3–1 Summer)	8750*	5280	990	240	900	1240	100
5 (3–1 Autumn)	11380*	5780	3220	420	1080	720	160
6 (7–3)	11720	5780	2880	260	1580	1120	100
Sig. diff. at 5% level	1131	1057	510	—	867	490	—

* These yields do not include a stubble yield of approximately 1000 lb of d.m. obtained from these treatments when the changeover from the 7–3 to the 3–1 system of management occurred.

Main features shown in Table 5 are:

(i) The highest annual yield was obtained from the pastures that were hard grazed in the winter but received the less intensive system of management for the remainder of the year. This was due to consistently high yields of ryegrass, white clover, and red clover.

(ii) Lowest annual yields were obtained from the pastures that were hard grazed either during the spring (treatment 3) or the summer (treatment 4) or were hard grazed throughout (treatment 1). In treatments 1 and 4 this was due to marked reductions in the yields of cocksfoot and red clover, particularly during the summer and autumn (Tables 3 and 4), and in treatment 3 to reductions in the yield of ryegrass and red clover during the spring and to a lesser extent of cocksfoot during the summer.

(iii) Yields obtained from treatments 5 and 6 were intermediate except for the yields of cocksfoot which were high during the summer and autumn (Tables 3 and 4).

Yields Obtained During the Winter and Spring of 1957

Table 6 shows total and species yields obtained during the following winter and spring of 1957 when all pastures were placed under the 7–3 system of management. Least significant differences at the 5% level are also shown.

The following are the main features in Table 6:

(i) Pastures hard grazed in the previous summer (treatments 1 and 4) yielded less total d.m. than treatments 2, 3, and 6 (the differences between treatments 1 and 4 and treatment 5 did not reach significance). The yield of cocksfoot from both treatments was

TABLE 6. WINTER AND SPRING YIELDS (1957)
(lb/ac)

Treatment	Total Yield	Species Yields					
		Ryegrass	Cocksfoot	Other Grasses	Red Clover	White Clover	Other Species
1 (3–1)	4540	2620	190	440	10	1050	230
2 (3–1 Winter)	5850	2140	2970	175	255	305	5
3 (3–1 Spring)	5480	1930	2730	220	110	460	30
4 (3–1 Summer)	4640	2210	870	285	70	1165	40
5 (3–1 Autumn)	5180	2310	2140	290	40	300	100
6 (7–3)	5960	1110	4060	240	195	345	10
Sig. diff. at 5% level	660	743	578	—	181	220	—

markedly reduced and, although white clover yields were high, the reduction was of sufficient magnitude to reduce total yields.

(ii) Highest yields were obtained from treatments 2 and 6. For treatment 6 this was due to a large increase in the yield of cocksfoot and in treatment 2 to high yields of both ryegrass and cocksfoot.

(iii) Yields obtained from treatments 3 and 5 were intermediate.

DISCUSSION

The results obtained illustrate the influence grazing management has on the growth and survival of pasture species and the effect of these on changes in botanical composition and ultimately on total herbage yields. They show that pasture productivity can be influenced by the severity or frequency of grazing, the effect being more dramatic at some times of the year than at others. As stated previously (Brougham 1959b), it is considered that the differences between the seasonal growth patterns produced by the treatments were determined by species tolerance to the different intensities of grazing and the modified action of weather factors on growth following changes in herbage cover and in botanical composition.

Hard grazings during the winter (treatment 2) encouraged the growth of all sown species. This was particularly evident for ryegrass in all seasons following the winter treatment, and for red clover in all seasons, especially the summer (Tables 3 and 5). The yields obtained from the frequently hard-grazed treatments during the first winter (1956) conform to the results obtained in the previous experiment and the explanation offered there in terms of light interception by the herbage of these treatments and possible limitations in the herbage measurement technique where d.m. losses through tissue decomposition were not accounted for in the laxly grazed treatments, would also apply to the present results.

In this locality, prevailing weather conditions are very seldom of sufficient severity to stop grass growth over the winter although temperatures and the amount of incident light energy are low enough to limit growth (Brougham 1959a). On the other hand, these growth factors can be modified by the amount of herbage cover left after grazing. Under more intensive grazing over the winter, light is allowed to penetrate to ground level so that growth and tillering in the grasses and growth of the clovers (Table 2) is encouraged. It is also possible that temperatures at the soil surface are higher on sunny days than in pastures where a cover of herbage is left after grazing, thus promoting more vigorous growth on these days. For these reasons one or more intensive grazings may be beneficial to growth over the winter as has been shown by the results obtained from this experiment and those obtained in a previous experiment (Brougham 1956) in which it was demonstrated that long spelling between grazings over the late autumn and winter adversely affected yields obtained under the practice of "Autumn Saved Pasture".

During the spring, frequent hard grazings markedly reduced total d.m. yields (Table 2) but because weather conditions (light, moisture supply, and temperature) are extremely favourable for growth during this season no long-term detrimental effects on species performance were recorded (Table 6) with the possible exception of red clover (Tables 3 and 5). This latter effect on red clover probably occurred towards the end of the spring because of its more rapid growth at that time. Because of the very favourable weather conditions during the spring most of the species used in pastures in New Zealand are able to tolerate most of the management systems applied. However, in view of the precise management required by a number of pasture species during the summer to ensure plant survival, and in particular by the species used in this experiment, management systems that are applied to pastures in the late spring are important.

The results presented in Table 3 show the immediate gain in d.m. yield (approximately 100%) and the enhanced performance of species, particularly of cocksfoot and red clover that is obtained by maintaining a cover of herbage on pastures throughout the summer. By comparing the yields obtained from treatment 6 with those from treatments 1 and 4 in the following autumn, winter, and spring (Tables 4 and 6), the pronounced long-term effect of this phase of summer management is also shown. These results suggest therefore that less intensive grazings of pastures in the late spring would further increase d.m. production.

During the summer, lack of available soil moisture often limits the growth of pasture species and in some years may be sufficient to cause plant death. In the previous study (Brougham 1959b) it was shown that better conservation of soil moisture was obtained in pastures where a cover of herbage was maintained compared with more intensive grazings where the soil was laid relatively bare after grazing. From the results obtained by Mitchell (1957) this was probably associated with increased availability of soil nitrogen. The operation of both these factors resulted in better individual plant performance and higher yields. This would also apply to the results obtained during the summer in this experi-

ment. Other factors considered in restricting growth in the previous experiment were higher soil temperatures under the more intensive system of management and less use of incident light energy because of the smaller amount of assimilating plant tissue left after grazing. Obviously these factors can also be considered in an explanation of the results of this experiment.

In the previous experiment it was suggested that under management systems where the growth of the legumes in the pasture was favoured during the summer and autumn, there was interspecies competition for light, resulting in base-shading of ryegrass plants and a reduction in rate of tillering. The results presented here suggest that, in addition to this, there is also interspecies competition between grass species during the summer and autumn. Tables 3 and 4 show that in treatment 6 in which cocksfoot and clover growth were favoured during the summer and autumn, ryegrass growth was restricted in the following winter and spring (Table 6). This suggests interspecies competition for light between cocksfoot and ryegrass resulting in a reduced rate of tillering in ryegrass, and very low yields of d.m. from this species. Cocksfoot yields during the winter and spring of 1957 on the other hand were high.

With favourable growing conditions over the summer and early autumn for species such as cocksfoot and red clover, and to a lesser extent white clover, and where management is such that the growth of these species is encouraged (treatments 2 and 6), the botanical composition of the pastures will obviously change to cocksfoot and clover dominance (Table 4). Although total yield of pastures hard grazed in autumn was some 20% less than that of the highest-yielding treatments (2 and 6), this management system did enable ryegrass to recover and produce relatively well in the following winter and spring (Table 6). When compared with treatment 6, which remained cocksfoot dominant during the last two seasons of measurement, the importance of autumn management is well shown. It is suggested that hard grazings in the autumn after pastures have been managed more leniently during the preceding summer serves to reduce competition for light between the summer-growing species and those in which growth is limited by the higher temperatures that prevail during the summer. This was particularly noticeable with the growth of the clover species in treatment 5 (Table 4) where a reduction in the yield of these species was associated with increased yield of ryegrass in the following seasons (Table 6). In practice, a single grazing in the late autumn may have the same effect (cf. treatment 2 in the winter of 1956). An added advantage would be that higher yields would be obtained from the legumes during the early autumn. However it is significant that the cocksfoot-dominant pastures during the winter and spring (treatment 6) yielded as much as any of the other treatments.

The comparison between treatments 1 and 6 at all seasons of the year shows that species tolerance to the two grazing systems and d.m. yields obtained follow the same pattern as those obtained in the previous study (Brougham 1959b). A similar explanation for these changes in botanical composition and yield would also apply to these results.

A number of conclusions can be drawn from the results presented here and in the previous study. It is considered that these conclusions are applicable to a number of farming areas in New Zealand because of the fact that consistent results have been obtained from the two experiments in different years, and that these are supported by observations made in other areas. Differences between treatments of up to 45% in the annual yield of herbage have been shown, and over shorter intervals differences of up to 140%. These differences are directly attributable to pasture management through the action of the grazing animal.

The results highlight the importance of summer grazing management to ensure persistency of pasture species so that high annual yields of herbage are maintained. To achieve this in normal seasons it is suggested that supplementary feeding by means of conserved grass products or crops is an integral part of the prevention of overgrazing in the summer and early autumn.

The second feature of the results obtained is the importance of intensive grazing in the late autumn to bring about a rapid change in botanical composition from dominance of summer-growing species to dominance of species that grow well in late autumn and winter. In this way the light-shielding clover canopy in particular is removed and growth and tillering of the grasses is encouraged. This new grass growth is then better able to withstand the extreme winter conditions than the older plants in the pasture (Beddows and Jones 1958).

The other feature of the results is the relatively high yield of herbage obtained from intensive grazings of pasture during the winter. In practice, pastures are normally spelled over this period and the feed thus conserved is fed out *in situ* to lactating animals in the late winter or early spring. From these results and those obtained earlier (Brougham 1956), long spelling of pastures over the late autumn, winter, and early spring results in reduced d.m. yields and poor recovery growth in the spring. Higher yields of herbage are obtained by one or more grazings during this period.

Finally, it has been shown that the system of management applied to pastures has a marked effect on species survival and performance, on altering botanical composition, and ultimately influencing the yield of d.m. obtained annually from pastures. In both experiments, it has also been shown that under management systems involving repeated overgrazing, particularly in the summer, the amount of herbage produced is severely reduced. The highest-yielding treatments produced enough dry matter for the feed requirements of at least one milking cow per acre, whereas herbage production from the lowest yielding treatments was very much in line with that normally approached on dairy farms throughout the country. When compared with estimates of potential annual production from pasture (Brougham 1959a), the need for a detailed revision of existing pasture-management procedures and of better-planned management systems in which the requirements of the soil, the pasture, and the animal are considered, should be obvious.

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A NOTE ON THE OCCURRENCE OF *Azotobacter beijerinckii* IN A MARKET-GARDEN SOIL

By D. J. Ross, Soil Bureau, Department of Scientific and Industrial Research, Wellington

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Summary

The nitrogen-fixing bacterium, *Azotobacter beijerinckii,* has been isolated from a market-garden soil at Lower Hutt. This is believed to be the first recorded occurrence of this organism in a New Zealand soil. *Azotobacter chroococcum* was not detected.

Introduction

Azotobacter species were not found in the soils of some New Zealand tussock-grasslands either before or after cultivation (Ross 1958, in press). These soils were of low natural fertility and the acidity of three of the four soils examined would not be expected to favour the growth of *Azotobacter chroococcum.* A more fertile market-garden soil of pH 6.8 has now been examined, but *Azotobacter chroococcum* has again been found to be absent. However, a nitrogen-fixing bacterium has been isolated and, from the study of its properties here recorded, classified as *Azotobacter beijerinckii.*

Materials and Methods

Soil

The market-garden soil (Waikanae silt loam, pH 6.8) was collected in February 1956 at a depth of 2–4 in. from a market garden at Lower Hutt (Map reference: 498340 on Sheet N160).

Isolation of Azotobacter

An enrichment technique was used with the following medium (Mulder 1950):—K_2HPO_4, 1 g; $MgSO_4.7H_2O$, 1 g; $CaCO_3$, 2.5 g; $FeCl_3.6H_2O$, 10 mg; $ZnSO_4.7H_2O$, 1 mg; $Na_2MoO_4.2H_2O$, 0.5 mg; glucose, 10 g; tap water, 1 l. The soil (1 g undried) was added to the medium (20 ml in 250 ml conical flask) and incubated at 24°c in the dark for 28 days. A loopful of the resulting growth was spread over plates of N-free* glucose agar and isolated colonies further sub-cultured and examined.

Media

N-free glucose agar refers to the above medium solidified with agar (1.5% w/v), and N-free glucose silica gel to the above medium solidified with silica gel, pH 7.5, prepared by the method of Taylor (1950).

In N-free benzoate agar, sodium benzoate replaced glucose in the above medium.

Nutrient agar and tryptone were Difco products.

* N-free media contain no added nitrogen compounds.

Incubation

All cultures were incubated in the dark at 24°C.

Biochemical Tests

Carbohydrate metabolism was determined in the above N-free medium, with the appropriate carbohydrate (1.0% w/v) replacing glucose, and phenol red as indicator.

Nitrate reduction was studied in the N-free glucose medium supplemented with KNO_3 (0.1% w/v). The test for nitrites was made with Griess-Illosvay reagent.

Gelatin tubes (10% w/v gelatin) were inoculated with a stab of the organism.

A lead acetate paper suspended in a test tube over a culture growing in tryptone-glucose medium was used for the detection of hydrogen sulphide.

Nitrogen Fixation

The medium of Jensen (1940) was used with the glucose (1.0% w/v) sterilised separately and then added to the basal medium.

Nitrogen fixation at different pH values was carried out in the following medium:—KH_2PO_4, 6.8 g (0.05 M); $MgSO_4.7H_2O$, 0.5 g; $FeCl_3.6H_2O$, 0.1 g; $Na_2MoO_4.2H_2O$, 0.05 g; $CaCl_2.6H_2O$, 0.05 g; mannitol, 10 g; distilled water, 1 l. The pH was adjusted with the glass electrode, before sterilisation, with 2N NaOH or 2N HCl.

Nitrogen was determined by the Kjedahl method as before (Ross 1958).

Glucose was estimated by the method of Somogyi (1952).

Results

Isolation of the Organism

From the initial plating of the enrichment culture many colonies of the bacterium here studied were apparent. No other species of *Azotobacter* were seen.

Cultural and Morphological Characters

On N-free glucose agar or silica gel after 7 days' incubation, colonies were round (5–8 mm diameter), entire, convex, glistening, opaque, cream coloured, and of slightly mucoid consistency.

On N-free benzoate (0.1% w/v) agar, slight growth resulted after 7 days and the colonies (0.5–1 mm diameter) were colourless and translucent. A few colonies were obtained when the concentration of benzoate was 0.5%, but not when 1.0%. Tchan (1953b) states that the growth of both *Azotobacter chroococcum* and *A. beijerinckii* is inhibited by benzoate at a concentration of 1.0% but not at 0.5%.

After incubation for 7 days on nutrient agar, growth was comparatively poor, the colonies being round (1–2 mm diameter), entire, flat, dull, translucent and nearly colourless.

Growth in tryptone (1% w/v)-glucose (0.5% w/v) medium consisted of a slight turbidity and deposit after 7 days, whereas a heavy turbidity and deposit, with no pellicle, resulted in the N-free glucose medium.

Cells harvested from N-free glucose agar after 7 days were Gram-negative, round to oval, 1.6–4.0 × 1.5–2.0 μ, with pronounced slimy envelopes. Many cells were in pairs or in chains up to 8 cells long. From no medium, at any growth stage, were motile cells noted.

Physiological Properties

No acid or gas from glucose, sucrose, or mannitol was noted after incubation for 28 days, the final pH of the glucose culture being 7.0.

Nitrate was not reduced after 7 days, gelatin was not liquefied and hydrogen sulphide was not produced after 14 days.

TABLE 1. Nitrogen Fixation and Glucose Consumption

Culture	Glucose utilised (mg/ml)	Nitrogen content of medium after growth (μg/ml)	Nitrogen fixed (μg/ml)	Nitrogen fixation (mg/g glucose utilised)
Control	0	4	—	—
Azotobacter beijerinckii	9.47	107	103	10.9

Results are the means of triplicate cultures incubated for 11 days in the medium of Jensen (1940).

The economy of nitrogen fixation, recorded in Table 1, is similar to that of most species of *Azotobacter* which fix about 11 to 15 mg nitrogen per gramme glucose consumed (Jensen 1955).

TABLE 2. Influence of pH on Nitrogen Fixation

pH (initial)	pH (after growth)	Nitrogen fixed (μg/ml)
4.5	4.2	0
5.0	4.7	2
5.9	5.6	37
6.5	6.4	70
7.0	6.9	69
7.5	7.5	1
8.0	8.0	0

Cultures were incubated for 14 days.

It is apparent from Table 2 that the organism is capable of growth under moderately acid conditions and fixation of nitrogen is still good at pH 5.6. Most strains of *A. chroococcum* cease to fix nitrogen in media below about pH 6.0 (Jensen 1955) but the lower limits for growth and nitrogen fixation of *A. beijerinckii* are about pH 5.1 (Jensen and Petersen 1955).

Classification

Although Tchan (1953b) and Jensen (1954) differ considerably in their classifications of the Azotobacteriaceae, the present isolate can be classified as *A. beijerinckii* under both schemes. Its lack of motility and cream pigment distinguish it from the motile, light-to-dark pigmented *A. chroococcum* (Jensen and Petersen 1955). The organism is readily distinguished from the acid-tolerant *A. macrocytogenes* on cultural and physiological characteristics. A comparison with Tchan's acid-tolerant strain of *A. beijerinckii* (Tchan 1953a) showed similarity in all properties.

It is believed that the presence of *A. beijerinckii* has not previously been recorded in a New Zealand soil.

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CHEMICAL INVESTIGATIONS ON BITTER PIT OF APPLES

II. THE EFFECT OF SUPPLEMENTARY MINERAL SPRAYS ON INCIDENCE OF PITTING AND ON CHEMICAL COMPOSITION OF COX'S ORANGE FRUIT AND LEAVES

By H. O. Askew, E. T. Chittenden, R. J. Monk, and Joyce Watson
Cawthron Institute, Nelson

(*Received for publication, 19 November 1959*)

Summary

Cox's Orange apple trees were sprayed about mid December and mid January with aqueous solutions of the acetates of calcium, magnesium, potassium, and sodium, and of superphosphate and borax to attempt to control bitter pit in the fruit in orchards on two differing soil types.

Analysis showed that use of the sprays altered the chemical composition of both fruit and leaves. Both fruit and leaves changed in composition and dry-matter content between mid January and harvest time in February or March, not only in respect of mineral and nitrogen content but also in the amounts of acids and sugars present.

In one season the calcium spray highly significantly reduced the amount of bitter pit at Annesbrook orchard, but was not significantly beneficial in the second season. On the Moutere Hills orchard in this second season calcium gave the best results, although not significantly so. Potassium in the three trials was very detrimental, significantly increasing the amount of pitting. Sodium and magnesium were detrimental also.

High dry-matter and ash contents and high values of the ratio K/Ca in the fruit were associated with increased amounts of bitter pit Chemical data for leader leaves appear to be more valuable than those for spur leaves for diagnostic purposes; high calcium content and high values for the Ca/K ratio are associated with a reduction in bitter pit in the fruit.

Introduction

In his fourth report McAlpine (1914-15) said of bitter pit, 'It is a problem of growth "gone wrong" and we must study the mechanism of growth "gone right" in order to control and prevent it.'

For many years attempts have been made by workers in several parts of the world to control the incidence of bitter pit in apples by manurial and cultural means without any definite success. The more direct trials of Garman and Mathis (1956), although open to criticism regarding experimental details, suggested that provision of supplementary supplies of minerals offered promise for attempts to control bitter pit in apples. In Part I (Askew *et al.* 1959) of this series of papers, data were presented on the chemical composition of fruit and leaves of Cox's Orange variety of apple, as a study in plant physiology, without any indication of the effect of chemical composition on storage

quality. This is now remedied by an account of trials using sprays of solutions of the acetates of calcium, magnesium, potassium, and sodium, and of superphosphate, on Cox's Orange trees at Annesbrook orchard of the Institute in two seasons and at a Moutere Hills orchard in one season. Data will be presented on the incidence of bitter pit in the fruit and on chemical composition of fruit and leaves following use of the above sprays.

Experimental

Soil Data

At Annesbrook the soil type is Ranzau gravelly silt loam; a recent soil derived from sedimentary rocks of the "Maitai Series" and greywacke gravels.

The Moutere Hills orchard is on Mapua sandy loam, a strongly leached soil derived from strongly weathered greywacke gravels.

Although no differential soil treatments were made on the experimental areas which received overall the normal annual topdressings of N, P, K by the owners of the orchards, soil samples were taken for chemical analysis from all the plots. This was done to have the assurance that no real differences existed between the plots which might influence the incidence of bitter pit.

The variations shown in the soil analysis between the plots on either orchard were no more than would be expected, and are unlikely to be sufficient to affect incidence of bitter pit. There is however a significant difference shown in the figures between the two orchards particularly in the exchangeable calcium and magnesium. The higher incidence of bitter pit on the Moutere orchard might be due to the lower base status of the soil.

The soil analyses of the two orchards are shown in Table 1 given as an average of all the plots.

Sprays

The basis of the calculations for the amounts of nutrient salts to be applied to the trees as sprays was as follows. The assumption made was that a tree carried 20 lb of leaves with a dry-matter content of 45%, giving 4050 g of dry matter. Analytical figures were taken at: Ca, 1.4%; K, 1.2%; Mg, 0.45%. In the flesh of the fruit the assumed figures were: Ca, 0.025%; Mg, 0.030%; K, 0.50%. Thus, on a tree basis, excluding fruit and twigs, the uptakes would be Ca, 57 g; Mg, 18 g; K, 85 g. The amounts of nutrient in the fruit are small for calcium and magnesium (less than 10 g), but for potassium it is 113 g, if the crop is 300 lb per tree of which 1/6th is dry matter. It was hoped to provide in the sprays approximately the same amounts of the several nutrients that would be present in the leaves. There would of course be appreciable loss of spray during application but that is unavoidable. The total quantities of chemical given below were applied in two sprays, using two gallons of water, without any spreader, per tree each time.

TABLE 1. SOIL ANALYSES

Orchard	Depth (in.)	Citric Sol.		pH	Org. C. (%)	N (%)	C/N Ratio	Loss on Ign. (%)	Cation Exchange					
		P_2O_5 (%)	K_2O (%)						B.E.C. (me.%)	T.E.B. (me.%)	B.S. (%)	CaO (me.%)	MgO (me.%)	K_2O (me.%)
Annesbrook	0–6	.043	.025	5.8	3.1	.23	13	9.6	21.8	16.8	77	14.0	2.3	.77
,,	6–12	.015	.008	5.4	2.2	.15	15	8.1	18.8	12.7	68	10.9	2.0	.19
,,	12–18	.008	.004	5.2	1.3	.10	13	6.5	14.5	8.4	58	6.9	1.8	.06
Moutere	0–6	.032	.013	5.6	1.6	.10	16	5.8	10.8	6.8	64	6.1	0.7	.36
,,	6–12	.002	.006	4.5	0.9	.05	18	5.5	12.9	1.6	12	1.4	0.3	.18
,,	12–18	.001	.004	4.5	0.6	.04	15	6.3	15.6	1.2	8	1.0	0.3	.09

Treatments

Calcium. 225 g dry calcium acetate per tree per season.
Magnesium. 160 g magnesium acetate (4 H_2O) per tree per season.
Potassium. 200 g potassium acetate per tree per season.
Sodium. 232 g sodium acetate (hydrated) per tree per season..
Phosphorus. 280 g superphosphate (to provide same amount of calcium as was present in the calcium acetate) per tree per season.
Boron. 0.2% solution of borax, 2 gallons per tree at each application.
Control. No sprays applied.

Superphosphate was used to supply both calcium and phosphorus —this treatment is called "phosphorus" later in this paper. It is a cheap source of a soluble calcium salt, and it was possible that the phosphorus, as well as the calcium, could be beneficial.

Boron, as borax, was used in the 1958-59 season only in the Moutere Hills orchard. In explanation of this treatment it may be stated that suggestions have been made that use of boron reduces the amount of bitter pit in fruit.

At Annesbrook there were four trees to each treatment employed there in 1957-58 but only three each in 1958-59 owing to collapse of trees in that area of the orchard. In the Moutere Hills orchard there were four trees for all seven treatments.

The nutrient materials were divided into equal parts to be applied in mid December and mid January. Actual dates of application were: in the 1957-58 season on 13 December and 17 January, and in the 1958-59 season on 18 December and 21 January at Annesbrook, and on 18 December and 20 January on the Moutere Hills orchard.

Composite samples from the trees of a given treatment were obtained in both seasons as follows:

Leaves: from leaders (bottom 5 leaves) and spurs, immediately before the mid-January spray and at harvest.

Fruit: immediately before the mid-January spray and at harvest. Average weights of the apples were recorded.

On the above materials percentages of dry matter in the fresh material, and total nitrogen, total ash, calcium, magnesium, potassium, sodium, phosphorus, glucose, fructose, and sucrose were determined, the results being expressed in the tables below as percentages of the dry matter. PH value of the dried powdered fruit was determined with a glass electrode on a mixture of 4 g of solid with 10 ml of water. Titratable acidity of the dried apples was determined on a water extract with titration to the phenolphthalein end point.

From each tree in the experiment 100 fruits of uniform size were taken for cool storage at 37–38°F; weights of these were recorded. In the 1957-58 season harvesting was done on 5 March and examination for incidence of bitter pit after storage on 1 July. In the 1958-59 season harvesting at Annesbrook was done on 4 February and grading for incidence of bitter pit on 18 and 19 May 1959, and at the Moutere Hills orchard fruit was stored on 3 February and examined on 20-24 April 1959. Grades of incidence of bitter pit were three, namely bad,

medium, and slight. In the bad grade pitting was very extensive and deep into the flesh; in the slight grade occasional areas of discoloured tissue, mainly near the surface of the fruit, were present; the medium grade was intermediate between these other two grades. The statistician gave a grading score of 10 for bad, 3 for medium, and 1 for slight; scores calculated from these were used in the statistical examination of the bitter-pit data.

RESULTS

Annesbrook Orchard

Incidence of Bitter Pit

Data for the weight of 100 apples from each tree in the experiments and for percentage of the fruits in the three categories of incidence of bitter pit, together with the total percentages, are set out in Table 2 for the 1957-58 season and in Table 3 for the 1958-59 season.

It will be seen that the samples per treatment were not strictly uniform in weight, although every endeavour was made to get them so. Variations are greater in the first than in the second season, owing to the very light crop that year. Incidence of bitter pit is greater the larger the size of the apple: this is well-known. But beyond this there are differences in amount of bitter pit which can only be explained as being due to tree individuality. The range in total percentages of affected fruits in 1958 was from one for the abnormally small fruit of tree 3 of the magnesium treatment to 82 for tree 4 of the sodium treatment. The smallest amount of pitting per treatment, an average of 6% of pitted fruit, was found for the calcium treatment, followed by phosphorus at 30%, while potassium at 60% was worst; the control (unsprayed) figure was 40%.

Making use of analysis of covariance to correct for variations in the weights of the fruits, and using the scoring system already mentioned, statistical examination of the data of Table 2 shows that the calcium treatment is highly significantly different from each of the others ($P = 0.01$), and the magnesium treatment to be almost significantly different from potassium ($P = 0.06$). Potassium was almost significantly different (at P 0.10) from sodium. In this trial the coefficient of variation was 20%.

In the 1958-59 season the effects of some of the sprays were very marked but the beneficial treatments were not so strongly shown as in the previous year. Weight of fruit and incidence of pitting are shown in Table 3.

Percentages of affected fruit range from 21 for calcium, 23 for control, 25 for magnesium, through 40 for sodium and 42 for phosphorus to 92 for potassium. As in the previous season there is in general increased pitting in the larger fruits; individual tree effects are again to be seen. Only one tree is reported upon in the potassium treatment, the other two trees having become unhealthy during the season and thus are abnormal.

TABLE 2. INCIDENCE OF BITTER PIT IN APPLES FROM SPRAY TRIAL (Annesbrook 1957–58)

Treatment	Tree No.	Weight of 100 Apples lb oz	Bitter Pit % Bad	Medium	Slight	Total
Calcium	1	24 – 12	0	4	3	7
	2	28 – 6	1	2	7	10
	3	27 – 0	0	1	2	3
	4	27 – 6	0	1	2	3
	Average	26 – 14	0	2	4	6
Magnesium	1	27 – 0	24	30	16	70
	2	26 – 8	3	14	21	38
	3	21 – 6	0	0	1	1
	4	26 – 5	1	8	19	28
	Average	25 – 5	7	13	14	34
Potassium	1	26 – 1	4	13	22	39
	2	25 – 2	1	20	35	56
	3	26 – 2	19	26	29	74
	4	25 – 10	22	24	26	72
	Average	25 – 12	11	21	28	60
Sodium	1	28 – 10	14	39	25	78
	2	25 – 6	3	4	11	18
	3	24 – 2	2	3	6	11
	4	27 – 14	24	35	23	82
	Average	26 – 8	11	20	16	47
Phosphorus	1	25 – 2	2	9	10	21
	2	28 – 0	3	18	20	41
	3	25 – 13	3	8	16	27
	4	26 – 10	0	5	27	32
	Average	26 – 6	2	10	18	30
Control	1	23 – 3	0	0	11	11
	2	26 – 13	8	19	13	40
	3	28 – 2	21	19	19	59
	4	26 – 4	7	15	27	49
	Average	26 – 1	9	13	18	40

Examination of the data shows that the potassium is significantly worse than the control and magnesium treatments ($P = 0.05$), while calcium is significantly better than sodium and phosphorus ($P = 0.05$), and highly significantly better than potassium ($P = 0.01$). Between the magnesium, sodium, phosphorus, and control treatments there are no significant differences. In this trial the coefficient of variation was 10%.

Calcium has therefore given the best result, although not to the same marked extent as in the first season. Supplying calcium as superphosphate has not been so beneficial as in the acetate form; there is evidence that the phosphate has given any added advantage. In both

TABLE 3. INCIDENCE OF BITTER PIT IN APPLES FROM SPRAY TRIAL (Annesbrook 1958–59)

Treatment	Tree No.	Weight of 100 Apples lb oz	Bitter Pit % Bad	Medium	Slight	Total
Ca	1	24 – 8	3	1	5	9
	2	26 – 5	6	5	11	22
	3	26 – 13	16	7	9	32
	Average	25 – 14	8	5	8	21
Mg	1	25 – 2	11	8	4	23
	2	23 – 8	11	11	8	30
	3	24 – 0	11	5	6	22
	Average	24 – 3	11	8	6	25
K	3*	25 – 8	74	12	6	92
Na	1	25 – 3	31	15	6	52
	2	24 – 7	12	8	5	25
	3	24 – 4	25	10	8	43
	Average	24 – 10	23	11	6	40
P	1	23 – 15	9	16	9	34
	2	24 – 0	20	13	6	39
	3	25 – 4	34	11	7	52
	Average	24 – 6	21	13	8	42
—	1	24 – 11	11	5	6	22
	2	24 – 6	13	6	6	25
	3	24 – 3	9	3	10	22
	Average	24 – 7	11	5	7	23

* The other two trees were in a very unhealthy condition at time of picking the fruit: consequently the data for them are not given.

seasons potassium has been especially detrimental to the fruit by encouraging increased incidence of pitting.

Chemical Data for Fruit and Leaves

Chemical data for fruit sampled just previous to the application of the January spray and at time of harvest have been obtained covering dry matter in the fresh material (Tables 4 and 6), titratable acidity and pH value (Table 5), and sugars (Tables 6 and 7).

In both 1958 and 1959 the percentage of dry matter in the fresh fruit was lowest at both samplings for the calcium treatment except for the potassium treatment in January 1959. In 1958 potassium gave the highest dry-matter figures.

For February 1959 two sets of figures are given. Fruit on this date was peeled, which explains the low dry-matter content. The unbracketed figures are averages for the whole fruit calculated from data such as those for the six sections in Table 6, while the bracketed ones are calculated from the three sections of the calyx half of the fruit. This is justifiable because incidence of bitter pit is greater in the calyx

TABLE 4. PERCENTAGES OF DRY MATTER ON FRESH BASIS OF FRUITS AT ANNESBROOK IN TWO SEASONS

Treatment	1958		1959	
	22 Jan.	5 Mar.	19 Jan.	4 Feb.
Calcium	13.0	10.3	14.9	13.3 (13.5)
Magnesium	13.4	14.2	15.7	14.3 (14.7)
Potassium	14.1	16.4	14.6	14.7 (14.9)
Sodium	13.7	14.7	14.9	14.6 (15.0)
Phosphorus	14.0	14.8	15.2	13.4 (13.5)
Control	—	13.5	15.3	14.4 (14.3)

half of the fruits; this bracketed figure has been used for correlating dry-matter content with the amount of bitter pit after storage. In further examination of the dry-matter figures of Table 6 it is seen that the top calyx and bottom stem sections have high dry-matter contents. This has been found to be generally true in the examination of many such sets of data. The high content at the calyx end can be correlated with the high incidence of pitting in this portion of the fruit.

TABLE 5. TITRATABLE ACIDITY AND pH VALUE OF DRIED FRUITS FROM ANNESBROOK ORCHARD

Factor Determined	Treatment	1958		1959	
		22 Jan.*	5 March	19 Jan.	4 Feb.
Titratable acidity	Calcium	5.5	2.9	5.9	6.1*
	Magnesium	3.9	2.5	6.0	5.0
	Potassium	5.6	2.6	5.3	6.2*
	Sodium	6.2	2.6	5.6	5.8
	Phosphorus	6.4	2.7	6.6	5.8*
	Control	—	2.9	5.7	5.4
	Average	5.5	2.7	5.8	5.7
pH value	Calcium	3.46	3.71	3.45	3.50
	Magnesium	3.49	3.98	3.48	3.48
	Potassium	3.47	4.02	3.39	3.58
	Sodium	3.49	4.00	3.36	3.52
	Phosphorus	3.48	4.00	3.39	3.54
	Control	—	4.07	3.40	3.42
	Average	3.48	3.96	3.41	3.51

* Dark colour of aqueous extract rendered end-point of titration difficult to determine.

Table 5 contains the data for titratable acidity and for the pH values of the dried fruit. In the 1958 season there is the normal large drop in acidity with approaching maturity of the fruit. In 1959 with the shorter period of time between the two dates of sampling the differences are much smaller. If the acidity figures are taken as measures of maturity then the fruit at harvest in 1959 was much less mature than in 1958. In some cases difficulty occurred in determining

TABLE 6. DRY MATTER ON FRESH BASIS, AND SUGARS IN HORIZONTAL SECTIONS OF APPLES FROM CONTROL PLOT, ANNESBROOK, 4 FEB. 1959
Chemical data expressed as percentages of dry matter

Section	Glucose	Fructose	Sucrose	Dry Matter
1 Stem end	4.4	28.8	16.9	14.6
2	4.8	25.9	16.2	14.3
3	4.7	26.7	14.9	14.6
4	5.5	19.4	15.9	13.3
5	4.4	27.0	15.0	14.1
6 Calyx end	4.9	21.3	12.9	15.5
Average	4.8	24.8	15.3	14.4

the end point of the titration and this may have a disturbing effect on any correlations that may be looked for between the values and incidence of pitting of the fruits.

The pH values of the dried fruits averaged 3.48 and 3.41 for the January samples of the two seasons, and 3.96 and 3.51 for the samples at harvest. In general the pH values increase with decrease in titratable acidity. The value of 3.71 for the calcium treatment in March 1958 is distinctly lower than all the others at this time; it is notable too that this treatment shows a smaller change in pH value with approaching maturity, although the titratable acidity is relatively high. This can only be explained by a difference in the mineral balance and buffering of the cell contents due to the use of the calcium spray. Similar relationships can be seen in other data of Table 5. In 1959 the pH value of the fruit at harvest was much lower than that in 1958.

Glucose, fructose, and sucrose contents of the six sections of fruit from the control plot at the February 1959 sampling are given in Table 6. These data are quoted to show the variations that have been found to occur generally in Cox's Orange fruits. Glucose content is usually low at the stem and calyx ends, and is high in the centre sections; fructose and sucrose are high at the stem end and low at the calyx; fructose is also high in the central sectors in a number of other samples. The average figures at the bottom of the glucose, fructose, and sucrose columns of Table 6 show that the data for the calyx end may differ somewhat from the whole-fruit averages.

Table 7 contains the average data for glucose, fructose, and sucrose contents of the fruits at the four sampling dates. In the first season, in the six weeks from 22 January to 5 March sucrose on the average increased sixfold and fructose content was nearly doubled; on the other hand the glucose content did not increase by very much. In the second season glucose contents were lower than in the first, but fructose and sucrose were higher at the January sampling in 1959 than in 1958; but at time of harvest fructose and sucrose were relatively low in 1959. Fructose levels at harvest in 1959 were not so much below the 1958 figures as were the sucrose figures; it will be noted that in 1958 with the more mature fruit sucrose content was nearly the same as the fructose, but in 1959 it was only about two-thirds of the fructose level.

TABLE 7. SUGARS CONTENTS OF APPLES FROM ANNESBROOK
Data expressed as percentage of dry matter

Sugar	Date	Treatment					
		Calcium	Magnesium	Potassium	Sodium	Phosphorus	Control
Glucose	22.1.58	8.4	8.5	7.8	8.2	8.7	—
	5.3.58	9.9	9.4	8.4	11.3	9.0	9.6
	19.1.59	5.2	8.2	7.8	5.1	5.5	5.5
	4.2.59	5.4	6.0	4.6	4.6	5.0	4.8
Fructose	22.1.58	14.1	14.3	14.2	17.6	15.5	—
	5.3.58	23.4	28.0	28.7	28.4	28.5	20.5
	19.1.59	21.1	23.3	22.1	21.9	22.7	21.3
	4.2.59	19.3	25.8	20.9	24.8	19.2	24.8
Sucrose	22.1.58	6.0	4.5	2.1	6.4	2.4	—
	5.3.58	28.7	22.4	26.9	22.3	27.0	26.0
	19.1.59	9.2	6.9	8.2	11.7	6.9	8.9
	4.2.59	13.6	13.9	12.3	17.1	13.5	15.3

There are some differences between the amounts of the three sugars which may be attributed to the treatments applied, and thence to the incidence of bitter pit in the fruit.

All the individual sections of the 1959 samples of fruit from the six treatments have been analysed for nitrogen and mineral content. A specimen set of data is given in Table 8 for apples from the control plot sampled on 4 February. Total ash, magnesium, potassium, and phosphorus contents were lower at the stem end than at the calyx end; on the other hand calcium and nitrogen were lower at the calyx end. It will be noted too that several constituents tend to maximum values in the centre sections; this has been found to be particularly true for potassium. Sodium content has not varied greatly from one part of the apple to another.

TABLE 8. PERCENTAGES OF DRY MATTER AND MINERAL AND NITROGEN CONTENTS OF HORIZONTAL SECTIONS OF APPLES FROM CONTROL PLOT, ANNESBROOK, 4 FEB. 1959
Chemical data expressed as percentages of dry matter

Section	Total Ash	Ca	Mg	P	K	Na	N
1 Stem end	1.17	0.024	0.023	0.035	0.33	0.007	0.50
2	1.21	0.018	0.024	0.038	0.47	0.011	0.43
3	1.15	0.021	0.024	0.040	0.47	0.006	0.53
4	1.38	0.018	0.028	0.046	0.55	0.007	0.55
5	1.24	0.017	0.025	0.047	0.57	0.007	0.45
6 Calyx end	1.23	0.016	0.025	0.039	0.46	0.008	0.42
Average	1.23	0.019	0.025	0.041	0.47	0.008	0.48

A matter of interest in respect of the data of Table 8, in view of the use made of average figures for minerals and nitrogen in Table 9, is that the average figures for the several constituents at the bottom of Table 8 approximate closely, except for nitrogen, to the composition of the top calyx section of the fruit.

TABLE 9. MINERALS AND NITROGEN CONTENTS OF FRUIT FROM SPRAY TRIAL AT ANNESBROOK, 1957-58
Chemical data expressed as percentage of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
22.1.58	Ca	2.20	0.045	0.047	0.082	0.83	0.014	0.91
	Mg	2.16	0.037	0.049	0.065	0.66	0.012	0.79
	K	2.20	0.040	0.045	0.075	0.79	0.015	0.76
	Na	2.36	0.042	0.053	0.083	0.64	0.029	0.85
	P	2.06	0.038	0.048	0.088	0.69	0.015	0.74
5.3.58	Ca	1.63	0.022	0.034	0.053	0.47	0.011	0.59
	Mg	1.62	0.024	0.035	0.061	0.62	0.016	0.62
	K	1.83	0.020	0.037	0.062	0.67	0.015	0.66
	Na	1.84	0.028	0.036	0.058	0.61	0.029	0.43
	P	1.63	0.026	0.031	0.053	0.57	0.015	0.43
	—	2.33	0.034	0.041	0.054	0.75	0.015	0.59

Mineral and nitrogen contents as average values from six sections, of fruit from the several treatments in the 1957-58 season are given in Table 9. Firstly it will be noted that in the six weeks between 22 January and 5 March the fruits have undergone the normal change bringing about lowered values for all the constituents estimated. There are clearly discernible effects from the use of the sprays. At the January sampling, calcium content has been increased by the calcium spray, but has been decreased by the magnesium and phosphorus ones. Magnesium content has been increased by both magnesium and sodium sprays. Phosphorus has been increased by the phosphorus, but decreased by the sodium and potassium sprays. Potassium was highest in the calcium and potassium treatments. Sodium content has been doubled by the sodium acetate spray, and nitrogen was highest in the calcium and sodium treatments. At the March sampling there are also effects to be seen from the application of the sprays in January. For example, the calcium spray has markedly reduced the potassium content of the fruit. Another matter of importance is the change in the ratio of calcium to magnesium between the two samplings. In January these two elements were present in approximately equal concentrations (as percentages), but in March, except in the control sample, magnesium is in proportionately much the greater amount. If the theory of Garman and Mathis (1956) is accepted then this state of affairs would

be important in respect of incidence of bitter pit. This change in the amounts of these constituents has been noted already in Part I of this series of papers (Askew *et al.*, 1959).

Table 10 contains a set of data for samples of the 1958-59 season. In this table also are the average weights of the fruits of the samples taken for analysis; it may be noted that the average weights calculable from the stored samples are not quite the same as the February data of Table 10. Between the two sampling dates the fruit increased in size from an average of 92 g to 109 g, while, as has already been seen, the average percentage of dry matter fell from 15.1 to 14.1 for the whole fruit and to 14.3 for the three sections at the calyx end of the fruit. Some part of this decrease will be due to the second series of fruits having been peeled before drying.

As in the first season so in the second season have the percentages of calcium and magnesium in the apples decreased with approaching maturity, with magnesium again being present in the larger amount at harvest. Compared with the first season, all constituents reported were usually present in lesser amount in the second. This applies to the January sampling, especially to the potassium and nitrogen figures, when the question of peeling the fruit did not enter. Also in the second season treatment did not have such a marked effect as in the first. The relatively small differences in chemical composition may thus be related to the smaller differences in the effects of the treatments on the relative incidence of bitter pit.

TABLE 10. Average Weight (g) per Fruit, and Minerals and Nitrogen Contents of Fruit from Spray Trial at Annesbrook, 1958–59

Chemical data expressed as percentages on dry basis

Date of Sampling	Treatment	Aver. Fresh Wt. g	Total Ash	Ca	Mg	P	K	Na	N
19.1.59	Ca	97.6	1.92	0.033	0.042	0.048	0.42	0.005	0.59
	Mg	92.0	1.64	0.036	0.037	0.048	0.42	0.007	0.51
	K	84.4	1.85	0.035	0.027	0.039	0.48	0.005	0.46
	Na	92.8	1.67	0.034	0.033	0.041	0.42	0.015	0.58
	P	90.0	1.77	0.035	0.034	0.055	0.41	0.008	0.59
	—	95.2	1.76	0.035	0.035	0.039	0.43	0.005	0.55
4.2.59	Ca	117.4	1.55	0.024	0.029	0.045	0.54	0.007	0.56
	Mg	104.0	1.29	0.020	0.025	0.047	0.49	0.007	0.48
	K	121.2	1.80	0.016	0.029	0.042	0.62	0.010	0.55
	Na	104.9	1.36	0.018	0.026	0.038	0.46	0.028	0.52
	P	100.2	1.55	0.020	0.030	0.047	0.57	0.013	0.59
	—	106.1	1.23	0.019	0.025	0.041	0.47	0.008	0.48

When the ratio of calcium to magnesium (Ca/Mg) is calculated for each of the treatments and for the four samplings, it is found that for the January samplings this ratio varies from 0.76 for the magnesium treatment to 0.96 for calcium in 1958, and from 0.80 for calcium to 1.30 for potassium in 1959; for the samples at time of harvest the ratio ranges from 0.54 for potassium to 0.84 for phosphorus in 1958, and from 0.55 for potassium to 0.83 for calcium. The unusually low figure for the potassium treatment in each season can be related to the very large amount of bitter pit found in the fruit from this treatment.

Another ratio, namely K/Ca, has also been used to see whether it bears any relationship to pitting of the fruit. In the 1957-58 season this ratio varied from 15.2 for sodium to 19.7 for potassium treatment in January, and from 21.4 for calcium to 33.5 for potassium treatment at harvest; variations from 11.7 for magnesium to 13.7 for potassium in January 1959, and from 22.5 for calcium to 38.7 for potassium in February were also obtained. Again the detrimental potassium treatment shows high values of this ratio.

Data for the percentages of dry matter on the fresh basis, and for the amounts of glucose, fructose, and sucrose in spur and leader leaves for the 1957-58 year are given in Table 11 and for 1958-59 in Table 12. In the first season, dry-matter contents of spur leaves varied from 33.2% to 37.9% in January, and from 35.2% to 42.1% in March;

TABLE 11. SUGARS AND DRY-MATTER CONTENT OF SPUR AND LEADER LEAVES FROM SPRAY TRIAL AT ANNESBROOK, 1957–58

Chemical data expressed as percentages of dry matter

Kind of Leaf	Treatment	Glucose %		Fructose %		Sucrose %		Dry Matter % (on fresh basis)	
		17.1.58	5.3.58	17.1.58	5.3.58	17.1.58	5.3.58	17.1.58	5.3.58
Spur	Ca	5.0	2.7	0.2	0.8	0.9	0.4	37.9	42.1
	Mg	4.9	2.8	0.2	1.0	0.5	0.2	37.1	38.5
	K	4.7	2.8	0.0	0.7	0.9	0.3	36.6	35.2
	Na	4.6	2.5	0.2	0.5	0.2	0.0	33.2	37.7
	P	4.7	2.6	0.1	0.7	0.2	0.2	37.5	35.8
	—	—	2.8	—	0.6	—	0.0	—	35.3
Leader	Ca	4.6	2.2	0.4	1.2	0.5	0.0	39.3	41.0
	Mg	5.5	2.6	0.3	1.1	0.1	N.F.	38.4	38.4
	K	4.6	2.5	0.3	0.9	0.9	0.2	37.8	39.5
	Na	4.6	2.6	0.0	0.8	0.5	N.F.	38.1	35.2
	P	4.7	2.8	0.5	0.5	0.2	0.0	38.1	33.8
	—	—	2.9	—	0.6	—	N.F.	—	37.2

N.F. means "not found".

TABLE 12. SUGARS AND DRY-MATTER CONTENT OF SPUR AND LEADER LEAVES FROM SPRAY TRIAL AT ANNESBROOK, 1958–59

Chemical data expressed as percentages of dry matter

Kind of Leaf	Treatment	Glucose %		Fructose %		Sucrose %		Dry Matter % (on fresh basis)	
		19.1.59	4.2.59*	19.1.59	4.2.59*	19.1.59	4.2.59*	19.1.59	4.2.59*
Spur	Ca	4.7	1.8	0.3	0.3	0.4	0.0	36.0	33.0
	Mg	5.4	3.9	0.6	1.1	0.2	0.1	39.6	34.2
	K	5.3	1.7	0.9	0.5	0.9	0.1	39.2	34.9
	Na	4.4	1.2	0.2	0.8	0.5	0.0	36.5	32.9
	P	4.3	2.0	0.4	0.0	0.4	0.1	38.2	32.5
	—	4.6	1.7	0.6	0.2	0.3	0.2	37.3	31.6
Leader	Ca	5.1	3.5	0.0	0.3	0.6	0.3	39.0	41.3
	Mg	5.9	2.0	0.7	0.0	0.8	N.F.	41.5	35.1
	K	5.8	2.0	0.6	0.1	1.3	0.4	38.3	36.8
	Na	4.9†	3.8	0.0	0.8	0.8	0.2	39.4	38.1
	P	4.9†	2.7	0.0	0.6	1.0	0.3	39.5	34.2
	—	5.4	2.1†	0.3	0.0	1.3	0.2	39.1	34.5

* Samples washed in dilute HCl
† Glucose greater than total reducing sugars
NF means "not found"

effects from treatments are not the same at the two dates of sampling. Owing to visible deposits of spray materials at sampling in February 1959 the leaves were washed in dilute hydrochloric acid and then rinsed in water. Dry-matter contents of the 1959 season's leaves are of the same order as those for 1958; the January samples, which were not washed, averaged 37.8% of dry matter for spur and 39.5% for leader leaves. It is interesting to note the high value for the dry-matter content of leader leaves from the calcium treatment in both seasons at harvest. The lowered dry-matter contents of 4 February 1959 cannot be attributed solely to the washing process because similar decreases occurred, somewhat erratically it is true, between the early and late samplings in the 1958 season.

Further, it is interesting to enquire if the dry-matter contents of the leaves and fruit are interrelated. In the 1958 season, increased dry-matter content of the leaves reduced the amount of dry matter in the fruit at both sampling dates, but in 1959 increasing dry matter in the leaves strongly increased the dry matter of the fruits in January, but exerted no influence at all in February. The effects seem therefore to be somewhat capricious.

Glucose contents of the two types of leaves were very similar in January in both seasons and did not show any seasonal differences.

Between January and the harvest date the glucose content in both kinds of leaves fell in each season to about half of the original values. On 4 February 1959 this effect may be accentuated by the washing treatment given at this time. Fructose and sucrose at corresponding dates were present in similar small concentrations in these leaves. It is noticeable that when sucrose is relatively high then fructose is low, and *vice versa*. Sucrose would appear to be very mobile because the low figures for the leaves correspond to times when rapid accumulation of this sugar is taking place in the maturing fruit. At this period fructose too is increasing in the fruit but the leaves do not seem to be drained of this sugar in the same way as is sucrose.

TABLE 13. MINERALS AND NITROGEN IN SPUR LEAVES FROM SPRAY TRIAL AT ANNESBROOK, 1957–58
Data expressed as percentage of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
22.1.58	Ca	7.58	1.96	0.34	0.20	1.03	0.040	2.61
	Mg	7.42	1.74	0.43	0.19	0.77	0.042	2.54
	K	8.05	1.71	0.36	0.20	1.40	0.041	2.61
	Na	8.13	1.89	0.38	0.21	1.22	0.106	2.70
	P	8.44	1.94	0.34	0.24	1.08	0.045	2.59
5.3.58	Ca	9.26	2.26	0.42	0.20	0.97	0.051	2.53
	Mg	9.00	2.16	0.50	0.18	0.80	0.055	2.56
	K	9.44	1.99	0.41	0.18	1.25	0.040	2.57
	Na	9.29	2.19	0.47	0.19	0.87	0.095	2.58
	P	10.25	2.41	0.41	0.22	1.13	0.048	2.56
	—	10.21	2.21	0.42	0.18	1.22	0.042	2.37

The minerals and nitrogen contents for the two dates of sampling in the 1957-58 season are given for spur leaves in Table 13 and for leader leaves in Table 14. There is an obvious increase in total ash, calcium, and magnesium contents, a relative constancy in phosphorus and sodium levels, and a tendency to reduction in the potassium and nitrogen contents between January and March. Somewhat similar changes in composition have occurred in both types of leaves. Comparing the data of Tables 13 and 14 it is found that spur leaves are richer in ash, calcium, magnesium, sodium, phosphorus, and nitrogen, but that they are poorer in potassium, than leader leaves. Each of the elements employed in the spray treatments has usually increased its content in both types of leaf at each sampling period, but has had little effect on the nitrogen content. Moreover, use of magnesium has reduced the calcium and potassium contents, and the potassium spray has lowered the calcium content. Sodium has been doubled in the spur, and trebled in the leader, leaves by the use of sodium acetate spray; this

TABLE 14. Minerals and Nitrogen in Leader Leaves from Spray Trial at Annesbrook, 1957–58
Data expressed as percentage of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
22.1.58	Ca	6.75	1.42	0.29	0.19	1.01	0.031	2.52
	Mg	6.04	1.09	0.43	0.17	0.83	0.029	2.37
	K	6.99	1.26	0.27	0.18	1.26	0.032	2.47
	Na	7.12	1.49	0.32	0.21	1.36	0.087	2.66
	P	7.65	1.52	0.24	0.23	1.42	0.038	2.60
5.3.58	Ca	7.53	1.38	0.27	0.18	1.06	0.041	2.52
	Mg	6.97	1.14	0.37	0.16	1.04	0.043	2.47
	K	8.23	1.42	0.34	0.17	1.22	0.033	2.43
	Na	8.01	1.35	0.31	0.17	1.03	0.138	2.65
	P	7.81	1.46	0.24	0.24	1.63	0.050	2.66
	—	6.96	1.23	0.24	0.16	1.49	0.040	2.55

treatment has also appreciably reduced the potassium contents of the leaves in March.

Similar data for the leaves of the 1958-59 season are set out in Tables 15 and 16. The reciprocal effects of addition of one nutrient on others is again noticeable: potassium has reduced both calcium and magnesium contents and magnesium has reduced potassium and calcium contents. On the other hand sodium has not reduced the calcium and has even increased the potassium content. It is interesting to note that the phosphorus spray, using superphosphate, has increased not only the calcium but also the potassium contents. Both spur and leader leaves of 4 February do not show any abnormal figures, so that the presumption is that the washing procedure has not affected their normal status.

If the data for corresponding leaves from the January samplings are compared there appear to be no great seasonal effects other than the relatively low potassium figures of the leader leaves in the second season; on 4 February 1959 the potassium content of the leader leaves was lower, but the calcium content was appreciably higher, than on 5 March 1958.

As with the fruit, the ratio Ca/Mg has been calculated for both types of leaves. The magnesium treatment reduced the value of this ratio in both spur and leader leaves, while the phosphorus treatment tended to the highest value, closely followed by the calcium treatment. When the ratio Ca/K in the leaves was calculated, low values were found, averaging about 1.2, for the potassium treatment, but it tended to be high for the calcium and magnesium treatments.

TABLE 15. MINERAL AND NITROGEN CONTENT OF SPUR LEAVES FROM SPRAY TRIAL AT ANNESBROOK, 1958–59
Data expressed as percentages of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
19.1.59	Ca	8.44	1.84	0.34	0.18	1.03	0.023	2.41
	Mg	7.50	1.72	0.40	0.18	0.65	0.022	2.44
	K	6.89	1.26	0.26	0.15	1.15	0.019	2.20
	Na	8.59	2.01	0.36	0.20	1.04	0.045	2.72
	P	9.11	2.06	0.34	0.23	1.36	0.023	2.49
	—	8.06	1.83	0.35	0.17	0.85	0.024	2.48
4.2.59*	Ca	8.47	2.04	0.38	0.19	1.02	0.024	2.51
	Mg	6.34	1.47	0.41	0.18	0.70	0.023	2.34
	K	6.85	1.28	0.28	0.15	1.39	0.020	2.40
	Na	7.75	1.83	0.36	0.19	0.89	0.120	2.37
	P	7.85	1.98	0.42	0.22	1.00	0.020	2.51
	—	7.91	1.85	0.37	0.16	0.76	0.020	2.39

* Samples washed in dilute HCl.

TABLE 16. MINERAL AND NITROGEN CONTENT OF LEADER LEAVES FROM SPRAY TRIAL AT ANNESBROOK, 1958–59
Data expressed as percentage of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
19.1.59	Ca	7.02	1.45	0.27	0.17	1.06	0.015	2.53
	Mg	6.31	1.44	0.33	0.16	0.62	0.018	2.22
	K	6.10	1.17	0.23	0.12	0.98	0.014	2.14
	Na	7.37	1.63	0.35	0.18	0.90	0.033	2.51
	P	7.70	1.71	0.31	0.21	1.06	0.016	2.66
	—	7.33	1.66	0.35	0.15	0.81	0.018	2.36
4.2.59*	Ca	7.36	1.75	0.32	0.17	0.71	0.017	2.51
	Mg	6.82	1.56	0.47	0.20	0.64	0.018	2.53
	K	7.32	1.40	0.28	0.17	0.21	0.017	2.27
	Na	7.32	1.75	0.37	0.18	0.76	0.088	2.48
	P	8.20	1.81	0.32	0.26	0.89	0.018	2.47
	—	7.60	1.89	0.40	0.16	0.76	0.022	2.48

* Samples washed in dilute HCl

Moutere Hills Orchard

In the 1958-59 season it was possible to arrange a further spray trial with Cox's Orange trees in an orchard on the Moutere Hills type of soil using four replications of uniform trees per treatment. The same elements at the same rates as at Annesbrook orchard were used, with the addition of a boron treatment, employing borax as 0.2%

TABLE 17. INCIDENCE OF BITTER PIT IN APPLES FROM SPRAY TRIAL AT MOUTERE, 1958–59

Treatment	Tree No.	Weight of 100 Apples lb oz.	Bitter Pit % Bad	Medium	Slight	Total
Ca	1	21 – 8	5	3	4	12
	2	21 – 6	4	2	8	14
	3	21 – 8	7	11	6	24
	4	22 – 0	43	20	5	68
	Average	21 – 9	15	9	6	30
Mg	1	23 – 3	25	7	10	42
	2	20 – 0	6	2	7	15
	3	21 – 8	17	20	10	47
	4	21 – 5	48	17	17	82
	Average	21 – 8	24	11	11	46
K	1	21 – 11	40	17	18	75
	2	22 – 6	30	13	17	70
	3	21 – 9	35	17	15	67
	4	21 – 14	33	17	15	65
	Average	21 – 14	34	16	16	66
Na	1	22 – 15	28	14	16	58
	2	22 – 10	29	17	14	60
	3	21 – 4	13	4	9	26
	4	21 – 12	41	21	13	75
	Average	22 – 2	28	14	13	55
P	1	20 – 7	9	13	13	35
	2	20 – 8	5	13	7	25
	3	20 – 10	14	10	11	35
	4	20 – 10	10	2	4	16
	Average	20 – 9	9	10	9	28
B	1	18 – 13	12	6	4	22
	2	22 – 10	31	22	14	67
	3	20 – 12	20	19	11	50
	4	22 – 5	22	4	16	42
	Average	21 – 2	21	13	11	45
—	1	21 – 9	10	2	15	27
	2	21 – 8	15	14	10	39
	3	21 – 5	4	1	6	11
	4	20 – 5	15	8	10	33
	Average	21 – 3	11	6	10	27

aqueous solution at 2 gal per tree at the same time as the other treatments were applied. Procedures for sampling and for storage of the fruit were precisely the same as in the Annesbrook trial. The analyses of soil samples from this Moutere Hills orchard have already been discussed.

Incidence of bitter pit and weight of 100 apples for each tree for the seven treatments are given in Table 17. In general there was little variation from tree to tree in the weights of the fruits, except that the phosphorus treatment is somewhat lower than the others. It will be noted that the potassium, sodium, magnesium, and boron treatments have appreciably more bitter pit than the control, calcium, and phosphorus ones. There is evidence, as at Annesbrook, of individual tree variation. Statistical examination of the data has shown that the control, calcium, and phosphorus treatments are lower than magnesium and boron at $P = 0.10$ approximately, and below potassium and sodium at $P = 0.05$. The greatest difference, between calcium and potassium, is almost equal to $P = 0.01$. The coefficient of variation in this trial was 12%. Therefore, although the calcium and phosphorus (supplying calcium also) treatments have not been beneficial, they at least have not been detrimental to quality of the fruit as have the other four treatments.

Variation in dry-matter content of the six sections of an apple are given for the control sample in Table 18. The low value at the calyx end does not always occur, but it did do so for several of the sets of sections from this experiment. At the stem end the dry-matter content was high in 6 out of the 7 treatments.

Percentages of dry matter in the whole fruits of 20 January and of peeled fruits of 3 February are given in Table 19; in this table the averages of the three sections towards the calyx end of the fruit of 3 February are given in brackets. These latter are used in preference to the figures for the whole fruit for the reason given when discussing the second Annesbrook experiment. An appreciable decrease in dry-matter content between the two sampling dates is evident.

TABLE 18. SUGARS IN HORIZONTAL SECTIONS OF APPLES FROM CONTROL PLOT, MOUTERE, 3 FEB. 1959
Data expressed as percentages of dry matter

Section	Glucose	Fructose	Sucrose	Dry Matter on fresh basis
1 Stem end	4.4	17.9	15.7	12.7
2	5.0	23.5	14.6	13.1
3	4.6	23.2	14.8	12.4
4	4.6	26.5	11.8	12.0
5	5.2	23.4	14.9	13.4
6 Calyx end	4.8	21.4	13.4	10.4
Average	4.8	22.6	14.2	12.3

TABLE 19. SUGARS AND DRY-MATTER CONTENT OF FRUIT FROM SPRAY TRIAL AT MOUTERE, 1958–59
Sugars expressed as percentages of dry matter

Treatment	Glucose		Fructose		Sucrose		Dry Matter on fresh basis	
	20.1.59	3.2.59	20.1.59	3.3.59	20.1.59	3.2.59	20.1.59	3.2.59
Ca	5.2	3.9	21.8	27.4	9.0	14.5	14.7	13.0 (12.9)
Mg	5.8	3.7	19.2	25.7	8.7	14.7	15.2	12.8 (12.9)
K	4.8	4.4	22.1	26.1	9.4	16.5	15.4	12.3 (13.3)
Na	7.0	4.3	21.8	26.4	7.4	14.9	15.3	12.0 (13.3)
P	6.0	4.7	23.3	27.3	5.0	14.8	15.6	13.5 (13.5)
B	5.6	5.7	25.4	23.9	9.0	14.4	15.0	11.7 (12.3)
—	6.7	4.8	21.7	22.5	4.4	14.2	15.6	12.3 (12.6)

Sugars contents of six sections of fruits from the control treatment of 3 February, that is at time of placing in cool store, are given in Table 18. As at Annesbrook, glucose and fructose were relatively low at either end of the fruit, and sucrose was low at the calyx end. If these data are compared with those in Table 6 for the Annesbrook fruit sampled on 4 February it is found that the glucose figures are very similar for the two sets of samples, but that fructose and sucrose are higher at Annesbrook than on the Moutere Hills orchard. For all three sugars the data for the calyx-end section are very similar at the two orchards.

Table 19 gives the average data for glucose, fructose, and sucrose contents, calculated from analysis of six sections with each treatment; there have been marked increases in the fructose and sucrose contents, but decreases in glucose, over the fortnight between the first and second sampling. At Annesbrook for the several treatments the glucose is somewhat higher, the fructose about 3% lower, and the sucrose just lower, than at the Moutere Hills.

Titratable acidity and pH value for the two sets of samples are given in Table 20. Free acidity has increased somewhat from 20 January to 3 February, which is unusual, and pH value has increased also. This latter effect must mean that the buffering of the constituents must be different at the two times of sampling; increased intake of potassium and other minerals could account for this.

As with the second season's data at Annesbrook so for the Moutere Hills trial is a specimen set of data, from the control plot, given for mineral and nitrogen contents of six sections of the fruit at harvest in February. Table 21 contains these data. Definite variations in concentration of the constituents, here expressed on the dry-matter basis, are to be seen. For all constituents except for sodium, and for phosphorus at the stem end, the lowest concentrations are at the stem and especially the calyx end of the fruit. Section 4 is notably high in

TABLE 20. FREE ACIDITY AND pH VALUE OF DRIED APPLE FROM SPRAY TRIAL AT MOUTERE, 1958–59
Free acid expressed as me. per 10 g of dry matter

Treatment	Acidity		pH Value	
	20.1.59	3.2.59	20.1.59	3.2.59
Ca	5.7	6.1	3.31	3.43
Mg	4.6	6.4	3.38	3.39
K	5.5	5.9	3.37	3.49
Na	5.0	6.0	3.41	3.48
P	4.9	5.4	3.43	3.47
B	5.1	5.3	3.38	3.52
—	5.2	5.4	3.36	3.49
Av.	5.1	5.8	3.38	3.47

all the constituents in all the treatments of this experiment, except for calcium being inconsistent. The magnesium treatment was unusual in that there were small variations only in the amounts of the elements in the several sections; the phosphorus treatment gave low potassium and nitrogen figures with a marked increase in phosphorus in the central sections. The Annesbrook data also show the same concentration of nutrients in the fourth (or sometimes fifth) section. An average figure is given for each constituent. This is the figure quoted for the control plot in Table 22 and similarly for the other plots mentioned in that table.

TABLE 21. PERCENTAGES OF DRY MATTER, AND MINERAL AND NITROGEN CONTENTS OF HORIZONTAL SECTIONS OF APPLES FROM CONTROL PLOTS, MOUTERE, 3 FEB. 1959
Chemical data expressed as percentages of dry matter

Section	Total Ash	Ca	Mg	P	K	Na	N
1 Stem end	1.49	0.019	0.022	0.050	0.51	0.011	0.37
2	1.55	0.030	0.025	0.045	0.64	0.009	0.43
3	1.49	0.031	0.030	0.048	0.63	0.010	0.44
4	1.70	0.028	0.030	0.070	0.77	0.011	0.54
5	1.61	0.026	0.031	0.049	0.53	0.010	0.42
6 Calyx end	1.35	0.020	0.027	0.038	0.47	0.014	0.41
Average	1.53	0.026	0.027	0.050	0.59	0.011	0.43

Average weights of fruits taken for analysis, and data for mineral and nitrogen contents of the fruits in January and February from the seven treatments are set out in Table 22. There is no effect of the treatment on the size of the fruits, but there has been some increase in

TABLE 22. MINERAL AND NITROGEN CONTENTS, AND AVERAGE FRESH WEIGHT PER FRUIT, FROM SPRAY TRIAL AT MOUTERE, 1958–59

Chemical data expressed as percentage of the dry matter.

Date of Sampling	Treatment	Aver. fresh wt. (g)	Total ash	Ca	Mg	P	K	Na	N
20.1.59	Ca	88.0	1.87	0.037	0.040	0.061	0.52	0.004	0.56
	Mg	76.8	1.90	0.039	0.040	0.059	0.49	0.005	0.46
	K	81.8	1.66	0.038	0.039	0.055	0.48	0.008	0.42
	Na	80.4	1.82	0.038	0.043	0.056	0.45	0.020	0.38
	P	80.4	1.62	0.037	0.039	0.044	0.52	0.005	0.42
	B	83.2	1.82	0.042	0.040	0.058	0.54	0.006	0.41
	—	86.4	1.63	0.039	0.040	0.047	0.42	0.004	0.39
3.2.59	Ca	94.7	1.50	0.023	0.029	0.046	0.55	0.008	0.51
	Mg	87.5	1.64	0.024	0.027	0.042	0.57	0.009	0.46
	K	90.2	1.64	0.023	0.031	0.049	0.65	0.016	0.41
	Na	87.6	1.53	0.019	0.022	0.035	0.41	0.034	0.41
	P	84.2	1.32	0.024	0.027	0.040	0.49	0.012	0.37
	B	87.0	1.68	0.028	0.030	0.043	0.58	0.014	0.39
	—	91.3	1.53	0.026	0.027	0.050	0.59	0.011	0.43

size, amounting to an average of 6.5 g between the two samplings. The fruits are somewhat smaller than those from Annesbrook. It will be remembered that the data for 3 February refer to peeled fruit. Except for the increased sodium content in the sodium treatment there was little effect on composition of the January fruit from the sprays applied on the previous 20 December. A further spray was applied on 20 January immediately after the samples were taken on that day and only a fortnight elapsed before the harvest date of 3 February. Except for potassium and sodium contents, and the low potassium figure where the sodium spray was used, there were no consistent variations from the control data following use of the sprays. The sodium treatment was unusual in that the calyx section of the fruit contained only 0.008% calcium and 0.015% magnesium, figures much lower than the averages for these constituents given in Table 22.

The Ca/Mg ratio for the fruits varied from 0.88 to 1.05 in January, and from 0.74 to 0.96 in February: it was therefore low throughout. In the January fruit the K/Ca ratio varied from 10.8 to 14.1, and in the February fruit from 20.4 to the very high value of 28.3 for the potassium treatment. The values of both of these ratios are similar to those of the Annesbrook fruit sampled at about the same time.

Data for the percentages of dry matter in spur and leader leaves, and for their glucose, fructose, and sucrose contents are shown in Table 23.

TABLE 23. SUGARS AND DRY-MATTER CONTENT OF SPUR AND LEADER LEAVES FROM SPRAY TRIAL AT MOUTERE, 1958–59

Chemical data expressed as percentage of dry matter

Kind of Leaf	Treatment	Glucose		Fructose		Sucrose		Dry Matter % on fresh basis	
		20.1.59	3.2.59*	20.1.59	3.2.59*	20.1.59	3.2.59*	20.1.59	3.2.59*
Spur	Ca	5.3	2.5	0.3	0.4	0.6	0.1	39.1	36.7
	Mg	5.2	2.4	0.4	0.5	0.0	0.2	38.3	36.3
	K	4.9	2.6	0.0	0.5	0.4	0.3	38.3	34.3
	Na	5.1	2.5	0.3	0.8	0.2	0.1	38.3	36.7
	P	5.1	2.4	0.3	0.5	0.3	0.4	39.9	35.0
	B	4.6	2.2	0.2	0.8	0.4	0.6	39.1	35.0
	—	4.8	3.3	0.6	0.4	0.3	0.6	37.0	35.2
Leader	Ca	5.4	2.7	0.0	0.9	0.5	0.0	41.9	44.5
	Mg	5.2	2.4	0.1	0.6	0.6	0.0	43.0	39.7
	K	5.1	2.6	0.0	0.3	0.2	0.4	42.8	41.4
	Na	5.2	2.1	0.2	0.6	0.4	0.1	41.1	41.9
	P	5.0	3.0	0.4	0.2	0.3	0.0	43.2	40.5
	B	5.4	3.8	0.4	0.0	0.0	0.4	44.3	39.6
	—	5.3	2.6	0.7	0.7	0.1	0.5	39.2	38.4

* Leaves washed with dilute HCl

As in the second Annesbrook experiment the levels of dry matter and of sugars are probably reduced at the February sampling owing to the washing treatment given to remove spray residues. In both sets of samples higher figures were found at the first date of sampling, and at both dates leader leaves were higher on the average than spur leaves. There was also a larger difference between the average values for spur leaves than for leader leaves, this being 3.0% as against 1.3%.

When the percentage of dry matter in the leaves was plotted against percentage of dry matter in the corresponding fruits, lower dry-matter contents of both spur and leader leaves in January accompanied increased percentages of dry matter in the fruit, but in February there appeared to be no relationship between the respective figures.

The sugar data of Table 23 show that the amounts of the several sugars in the Moutere Hills leaves corresponded closely to those found on the different soil type at Annesbrook. Only relatively small differences in sugar content with spray treatment were found.

Total ash, mineral, and nitrogen data for the spur leaves are given in Table 24 and for leader leaves in Table 25 for the January and February samplings. Use of the calcium spray has increased the calcium but decreased the magnesium contents in both types of leaves in both

sets of samples. The phosphorus spray, providing both phosphorus and calcium, has increased the contents of both these elements in the leaves, but has decreased the amount of magnesium present in February. Potassium and sodium treatments have markedly increased the levels of the respective elements. The data also show an increased sodium content following application of borax. Similar effects from the sprays were obtained with the leader leaves as shown in Table 25.

TABLE 24. Mineral and Nitrogen Content of Spur Leaves from Spray Trial at Moutere, 1958–59
Data expressed as percentage of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
19.1.59	Ca	6.95	1.27	0.23	0.19	1.45	0.010	2.28
	Mg	7.03	1.29	0.24	0.19	1.43	0.010	2.42
	K	6.20	1.06	0.21	0.20	1.43	0.013	2.53
	Na	6.01	1.05	0.31	0.19	1.13	0.046	2.24
	P	6.81	1.21	0.26	0.23	1.34	0.010	2.35
	B	6.98	1.17	0.26	0.21	1.44	0.010	2.37
	—	6.80	1.13	0.25	0.20	1.32	0.010	2.23
3.2.59	Ca	8.03	1.55	0.23	0.21	1.31	0.018	2.36
	Mg	8.19	1.41	0.41	0.19	1.46	0.016	2.20
	K	7.80	1.10	0.28	0.20	1.79	0.015	2.22
	Na	7.10	1.22	0.30	0.19	1.28	0.093	2.18
	P	8.28	1.31	0.24	0.26	1.55	0.014	2.25
	B	7.43	1.23	0.28	0.19	1.49	0.024	2.27
	—	7.75	1.24	0.27	0.20	1.58	0.014	2.29

When the data of Tables 24 and 25 were compared with the corresponding data of Tables 15 and 16 for spur and leader leaves from Annesbrook orchard in the 1958-59 season, calcium, magnesium, and sodium were usually higher at both samplings at Annesbrook than at the Moutere Hills orchard; on the other hand potassium was low at the former orchard. Nitrogen was also higher at Annesbrook, especially in February. Phosphorus content was slightly higher in the Moutere orchard.

The Ca/Mg ratios for the spur leaves varied in January from 3.4 for sodium to 5.5 for calcium treatment, and in February from 3.4 for magnesium with calcium again high at 6.7. These cover ranges similar to those of the corresponding dates at Annesbrook. In the leader leaves the ranges were somewhat wider, especially in February, being for the two samplings 3.7 to 7.4 and 3.6 to 9.2; in both cases magnesium gave the lowest and calcium treatment the highest figures. The low values were similar to, but the high ones were much above, those at Annesbrook.

TABLE 25. MINERAL AND NITROGEN CONTENT OF LEADER LEAVES FROM SPRAY TRIAL AT MOUTERE 1958–59
Data expressed as percentage of dry matter

Date of Sampling	Treatment	Total Ash	Ca	Mg	P	K	Na	N
19.1.59	Ca	6.71	1.11	0.15	0.20	1.50	0.010	2.39
	Mg	6.50	1.03	0.28	0.19	1.35	0.009	2.30
	K	5.70	0.82	0.16	0.20	1.47	0.007	2.42
	Na	5.79	0.91	0.22	0.21	1.16	0.037	2.30
	P	6.51	1.11	0.20	0.25	1.34	0.009	2.46
	B	6.56	1.05	0.20	0.23	1.30	0.010	2.45
	—	6.79	1.09	0.18	0.24	1.28	0.010	2.31
3.2.59	Ca	6.88	1.29	0.14	0.20	1.31	0.018	2.34
	Mg	6.72	1.19	0.33	0.21	1.31	0.013	2.62
	K	6.73	0.96	0.18	0.21	1.67	0.018	2.56
	Na	5.97	0.99	0.20	0.22	1.21	0.089	2.39
	P	7.13	1.16	0.17	0.25	1.29	0.018	2.58
	B	6.80	1.09	0.22	0.22	1.39	0.023	2.56
	—	7.23	1.11	0.22	0.21	1.22	0.011	2.53

The Ca/K ratios at both dates of sampling were distinguished in both spur and leader leaves for their very low values with potassium treatment; these values were 0.74 and 0.61 for spur and 0.56 and 0.57 for leader leaves at the January and February samplings. These may be compared with the highest values of 0.93 and 1.18, and 0.85 and 0.98 respectively for spur and leader leaves.

DISCUSSION

It is pertinent to inquire whether any of the analytical data presented in preceding tables can be related to the incidence of bitter pit in the fruits. For this purpose the total percentages of affected fruits have been graphed against the particular constituent under examination. From the graphs so obtained linear correlations have been sought; it is not impossible that non-linear correlations could have been obtained in some cases where the data seemed to be somewhat scattered for linearity. It is also probable that some of the trends found may not be statistically significant. A table (Table 26) giving the results has been prepared to cover the two seasons at Annesbrook and the one season at the Moutere Hills orchard at each sampling date. In this table the + sign means that an increase in concentration of the constituent, or in value of the ratio, accompanies increased incidence of bitter-pit in the fruit; the — sign means increased value is associated with decreased pitting of the fruit; the 0 sign means that

there is no relationship discernible between the quantities being compared; a double sign means that there is a close relationship between the data under comparison, and a ? sign means that there is a doubtful relationship, possibly only a tendency in the direction indicated. The comparisons have been made for the fruit and for the spur and leader leaves separately.

For the fruit, thirteen comparisons are set out in Table 26. It will be seen that many of the correlations found are not consistent as

TABLE 26. SUMMARY OF CORRELATIONS BETWEEN CONSTITUENTS OF FRUIT AND LEAVES, AND RATIOS BETWEEN CONSTITUENTS, AND INCIDENCE OF BITTER PIT IN COX'S ORANGE APPLES

Part of Plant	Constituent or Ratio	Annesbrook 1958		Annesbrook 1959		Moutere 1959	
		Jan.	Mar.	Jan.	Feb.	Jan.	Feb.
Fruit	D.M. on fresh basis	+	+	+	+	+	+
	Glucose	0	0	0	—	—	—?
	Fructose	+?	+	0	0	0	—
	Sucrose	+	+	0	—	+	++
	Titratable acidity	—	—	—	0	0	+
	pH value	0	+	—	+	0	0
	Total ash	0	+	0	+	0	+
	Ca	—	+	0	—	—	—
	Mg	+	+	—	0	0	0
	K	—	+	0	0	—	0
	P	0	++	0	0	0	0
	Ca/Mg	0	0	+	—	0	—
	K/Ca	0	+	0	++	0	+?
Spur leaves	D.M. on fresh basis	=	—	+?	+	—	0
	D.M. in fruit	—	—	++	0	—	0
	Total Ash	+	+?	—?	—	—	—
	Ca	—	—	0	—	—	—?
	Mg	+?	0	—	—	0	0
	K	+	+	0	+	0	0
	P	0	—	0	0	0	0
	Ca/Mg	—?	—	0	—	0	—
	Ca/K	0	0	—	=	0	—
	Sugars	0	0	0	0	0	0
Leader leaves	D.M. on fresh basis	=	—	—	+	0	0
	D.M. in fruit	—	—	++	0	—	+
	Total ash	+?	+?	0	0	-	—
	Ca	0	—	—	—	=	=
	Mg	0	+	—	—	0	0
	K	+	+	0	++	0	+
	P	0	0	0	0	0	0
	Ca/Mg	0	0	0	0	—	—
	Ca/K	=	—	—	=	=	—
	Sugars	0	0	0	0	0	0

\+ Means that an increase in the constituent or ratio accompanies increased incidence of bitter pit in the fruit.
— Means that an increase in the constituent or ratio accompanies decreased incidence of bitter pit in the fruit.
0 Means that there is no relation to incidence of bitter pit in the fruit.
? Means that there is a tendency only.
A double sign means that a very close relationship exists between the quantities.

to direction; thus fructose level may be associated with either a decrease or an increase in incidence of pitting. Other inconsistencies are seen for calcium, magnesium, and potassium contents in relation to degree of pitting of the fruit. Of the consistent relationships that of increasing incidence of pitting with increased dry-matter content of the fruit is confirmatory of early observations made by McAlpine (1911-12, p. 44). Another of McAlpine's observations that pitted fruit was lower in acidity than normal fruit has not been consistently supported by the present data, but his (*loc. cit.* p. 45), and De Long's (1936), observations that high ash content is associated with increased pitting of the fruit has been confirmed. The claim of De Long (1936) for decreased calcium content as an accompaniment to increased severity of pitting (in Fallawater and Stark varieties) has however not been consistently found, although there is a tendency in that direction. Magnesium, potassium, and phosphorus content are apparently not related to the incidence of pitting, but the ratio Ca/Mg, as claimed by Garman and Mathis (1956) may be, and the ratio K/Ca certainly seems to be, so related at time of harvest even if not at the January sampling. Sugars contents do not show any consistent relationships in the present series of samples to incidence of pitting in the fruit.

In summary therefore, for diagnostic purposes, high percentages of dry matter in the fresh fruit and of total ash in the dry matter, and a high value of the ratio K/Ca, appear to be promising. At the present time it would not be safe to set limits for these in estimating the potential incidence of bitter pit in Cox's Orange fruits.

In Table 26 the sugars contents for the leaves have not been set out separately for glucose, fructose, and sucrose as they were for the fruits. Each sugar was examined separately to see if the levels in the leaves were related to degree of pitting of the fruit. No relationships were found. It was of interest to see whether the dry-matter content of the fresh leaves bore any relationship to the dry-matter content of the fresh fruits; no consistent relationships were found. A consistent relationship would have been very valuable because it is very much easier to determine the dry-matter content of leaves than of fruits. However, a further line of attack is available through the dry-matter content of the fresh leaves to see if that is related to incidence of pitting in the fruit grown on the same tree. As Table 26 shows again, inconsistent relationships were found with both spur and leader leaves; in general however the two types of leaf gave the same indication at a given sampling period. Ash content was not so indicative in leaves as in fruit, and again magnesium and phosphorus contents were not related to the quality of the fruit. Calcium content, especially of the leader leaves, was inversely, and potassium content was directly, correlated with incidence of bitter pit in the fruit. The Ca/Mg, as with the fruit, was not valuable for correlation, but the Ca/K ratio, especially with the leader leaves, was strongly correlated with pitting, increase in the value of the ratio accompanying reduction in incidence of pitting.

Summarising the leaf data, it may be said that high values for the calcium content and for the ratio Ca/K, and relatively low values for the potassium level, are desirable if incidence of bitter pit is to be low.

The question may be raised as to the propriety of making such full chemical examinations of the fruit and leaves sampled some time before harvest or of such detailed inquiry into the relationships of these data to the incidence of bitter-pit in fruit harvested at some later date. In reply it should be pointed out that any early information that will lead to knowledge of the possible incidence of bitter pit later in the season will be of value, because it may lead to a decision being made as to date of harvest or to a prediction as to the probable incidence of bitter-pit in the fruit in that particular season. Such information would be of inestimable value.

The data from the spray trials recorded in this paper show that the use of supplementary sprays of the main elements to be found in apple fruits and leaves can modify their chemical composition and can alter the susceptibility of the fruits to incidence of bitter pit. From the data of this paper it would seem that the inorganic constituents are of more importance than the sugars in deciding the storage quality of the fruit, although it may well be true that a certain level of sugars is desirable for good quality.

From the information available from the work of Garman and Mathis (1956) with other varieties of apple, and from the present field trials, the use of sprays of calcium salts appears to be a promising method of controlling the incidence of bitter pit in Cox's Orange fruits.

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CORRIGENDA

p. 146, 2nd line from bottom, *for* there is evidence *read* there is no evidence.

p. 158, Table 17, K Treatment, last column, *for* 70 *read* 60.

CHEMICAL INVESTIGATIONS ON BITTER PIT OF APPLES

III. CHEMICAL COMPOSITION OF AFFECTED AND NEIGHBOURING HEALTHY TISSUES

By H. O. Askew, E. T. Chittenden, R. J. Monk, and Joyce Watson, Cawthron Institute, Nelson

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Summary

Discoloured tissue and neighbouring apparently healthy tissue from Cox's Orange fruits affected with bitter pit have been analysed.

Discoloured tissue was higher in total ash, calcium, magnesium, potassium, sodium, phosphorus, and nitrogen than neigbouring healthy tissue. Magnesium content of the former was four times that of the latter. While healthy tissue was generally similar in mineral composition to that of whole peeled fruits at time of storage, the former was poorer in calcium. There is evidence of general migration of minerals and nitrogen from the healthy to the affected tissues.

Tissue from the affected areas was very low in sucrose but was well supplied with glucose and fructose. It was richer in glucose than the neighbouring healthy tissue. Reducing sugars increased markedly during storage. Apparently healthy tissue was comparable in sucrose content to that of the whole fruit at time of harvest. Starch content of affected tissue was 0.81% and 1.17% at two orchards, while accompanying healthy tissue showed only 0.19% and 0.13% respectively.

Total ash content was significantly positively related to increasing incidence of bitter pit. High values of the ratios Mg/Ca, K/Mg, K/Ca, and K/N accompanied high incidence of bitter pit in the fruit.

Introduction

The appearance of the necrotic tissue in apples affected by bitter pit prompts the inquiry whether that tissue will be of significantly different chemical composition from the surrounding apparently healthy tissue.

Analysis of whole apples by De Long (1936) and Garman and Mathis (1956) has shown that affected apples differ somewhat in chemical composition from normal ones. Moreover Garman and Mathis (1956) have analysed pitted tissues removed from affected Baldwin fruits and found that they were high in potassium, magnesium, phosphorus, and boron, when the data were expressed on the dry basis. McAlpine (1911-12, p. 45) quotes data for the Lord Wolseley variety, and finds that the pitted parts are high in ash and reducing substances but low in acid and sucrose.

A characteristic of pitted tissue is the constant presence of starch grains in the cells. This was early noted by McAlpine (1911-12) and has been reiterated by Carne (1948) in the following words, "Pit lesions always contain starch". There do not however appear to be any data on the actual percentage of starch in the lesions and in the neighbouring apparently healthy tissue.

In view of these investigations and because of the intrinsic interest in such data for Nelson fruit, a number of isolations of pitted tissues have been made from affected fruits in the cool-storage trials described in Part II of this series of papers (Askew *et al.* 1960). The chemical data obtained on this material and on healthy tissue from the same fruits are presented in this paper.

Experimental

In 1958, discoloured tissue was obtained from a storage trial with Cox's Orange fruit from three commercial growers. Healthy tissue was taken from other parts of the apples, but was not necessarily adjacent to the pitted tissue; some of it came from nearer the stem end, because in this part of the fruit pitting is usually much less pronounced than at the calyx end. In 1959 a great amount of detailed work was entailed in getting apparently healthy tissue from beside the pitted portions. Moreover, owing to the tree individuality that has been noted in the cool-storage trials (Askew *et al.* 1960), affected and healthy tissue has been taken in some instances from individual trees as well as composite samples from the remaining trees of a given treatment.

Glucose, fructose, and sucrose, as well as mineral and nitrogen contents, have been determined, the data obtained being expressed on the dry basis. The methods used were those described previously (Askew *et al.* 1959). In addition, starch has been determined using perchloric acid extraction (Pucher *et al.* 1948).

Results

Minerals and Nitrogen

In the 1958 season, pairs of samples of affected and healthy tissues from three orchards on the Moutere Hills soil type were analysed. The healthy tissue was taken from any part of the fruits and was not necessarily adjacent to affected areas. The data obtained showed that affected tissue was higher in ash, calcium, phosphorus, and especially magnesium, content than healthy tissue. Magnesium was doubled or quadrupled in the pitted material. Potassium, sodium, and nitrogen did not vary consistently.

A much wider range of paired samples was obtained in the 1959 season from the spray trials, comprising three pairs from Annesbrook orchard and eleven pairs from the Moutere Hills orchard. In both groups samples did not always cover all the trees used in a particular spray treatment. Thus at Annesbrook orchard the material for analysis was taken from all three trees in the sodium and phosphorus treatments, but from only one tree, with very badly affected fruit, in the potassium treatments. This is shown in Table 1 by the figures beside the appropriate treatment. From the four trees in each treatment at the Moutere Hills orchard separations have been made in the calcium, magnesium, sodium, and boron treatments; the figures against the treatment represent the trees from which the samples were taken. These separations were made by reason of differences in incidence of bitter pit, sometimes more severe and sometimes less severe, than was found

for the other three trees. The sample material was obtained at the time of making the counts of affected fruits after holding in cool store. The Moutere Hills fruit was in store from 3 February to 20-24 April, and that from Annesbrook orchard from 4 February to 18-19 May. Annesbrook fruit was therefore in cool store about 25 days longer than that from the Moutere Hills (Askew *et al.* 1960).

In Table 1, which contains the analytical data for the Annesbrook samples, and in Table 2, which deals with the Moutere Hills orchard, are also given the percentages of affected fruits for the trees whose numbers appear opposite the stated treatment. In these tables the data marked "original" refer to the composition of the whole fruit taken at the same time as the fruit was picked for storage; where two sets of affected and healthy fruit are given, the "original" figures refer to a composite sample of whole fruits covering all the trees in the specified treatment and not to whole fruit from the tree or trees concerned.

TABLE 1. PERCENTAGES OF MINERAL AND NITROGEN ON D.M. BASIS OF AFFECTED AND HEALTHY TISSUE FROM FRUITS FROM ANNESBROOK, 1959

Treatment	Status	Bitter Pit %	Total Ash %	Ca %	Mg %	P %	K %	Na %	N %
Na (1–3)	Pitted	40	2.46	0.033	0.14	0.065	0.64	0.042	0.66
Na (1–3)	Healthy	—	2.01	0.020	0.035	0.056	0.77	0.048	0.70
Na (1–3)	Original	–	1.36	0.018	0.026	0.038	0.46	0.028	0.52
K (3)	Pitted	92	3.15	0.040	0.14	0.11	1.27	0.020	0.70
K (3)	Healthy	—	2.56	0.019	0.034	0.066	1.02	0.018	0.85
K (3)	Original	—	1.80	0.016	0.029	0.042	0.62	0.010	0.55
P (1–3)	Pitted	42	2.81	0.040	0.14	0.088	0.78	0.011	0.69
P (1–3)	Healthy	—	2.13	0.019	0.037	0.057	0.82	0.015	0.68
P (1–3)	Original	—	1.55	0.020	0.030	0.047	0.57	0.013	0.59

In Table 1 there is very distinct evidence of a concentrating action having taken place by reason of the relatively high ash, calcium, magnesium, and phosphorus figures for the pitted tissue; potassium, sodium, and nitrogen behaved somewhat irregularly, when compared with the data for the apparently healthy tissue. When compared with the "original" figures, which refer to whole peeled fruits, the movement of all the nutrient materials reported appears to have been appreciable.

The data of Table 2, which refer to a wider range of treatments and give figures for individual trees, showed many of the same characteristics as those of Table 1. Ash, calcium, magnesium, and phosphorus were again high in pitted material and, in addition,

TABLE 2. PERCENTAGES OF MINERAL AND NITROGEN ON D.M. BASIS OF AFFECTED AND HEALTHY TISSUE FROM FRUITS FROM MOUTERE, 1959

Treatment	Status	Bitter Pit %	Total Ash %	Ca %	Mg %	P %	K %	Na %	N %
Cont. (1–4)	Pitted	27	2.69	0.038	0.14	0.086	0.71	0.009	0.57
Cont. (1–4)	Healthy	—	1.78	0.012	0.029	0.040	0.56	0.008	0.46
Cont. (1–4)	Original	—	1.53	0.026	0.029	0.050	0.59	0.011	0.43
Ca (1–3)	Pitted	17	2.41	0.035	0.11	0.087	0.74	0.010	0.53
Ca (1–3)	Healthy	—	1.73	0.015	0.029	0.056	0.78	0.010	0.50
Ca (4)	Pitted	68	2.94	0.034	0.14	0.088	0.93	0.010	0.56
Ca (4)	Healthy	—	1.72	0.015	0.027	0.031	0.46	0.008	0.48
Ca	Original	—	1.50	0.023	0.029	0.046	0.55	0.008	0.51
Mg (1–3)	Pitted	35	2.63	0.033	0.14	0.082	0.78	0.009	0.53
Mg (1–3)	Healthy	—	1.61	0.011	0.029	0.041	0.66	0.008	0.46
Mg (4)	Pitted	82	3.04	0.027	0.14	0.105	1.16	0.011	0.68
Mg (4)	Healthy	—	1.95	0.014	0.029	0.053	0.85	0.010	0.52
Mg	Original	—	1.64	0.024	0.027	0.042	0.57	0.008	0.46
K (1–4)	Pitted	66	2.71	0.029	0.14	0.082	0.96	0.019	0.47
K (1–4)	Healthy	—	1.99	0.016	0.030	0.051	0.78	0.016	0.50
K (1–4)	Original	—	1.64	0.023	0.031	0.049	0.65	0.016	0.41
Na (1, 2, 4)	Pitted	64	2.32	0.033	0.14	0.083	0.75	0.087	0.45
Na (1, 2, 4)	Healthy	—	1.74	0.018	0.031	0.053	0.70	0.067	0.43
Na (3)	Pitted	26	2.51	0.039	0.13	0.084	0.69	0.080	0.46
Na (3)	Healthy	—	1.62	0.018	0.027	0.057	0.75	0.067	0.40
Na	Original		1.53	0.019	0.022	0.035	0.41	0.034	0.41
P (1–4)	Pitted	28	2.30	0.032	0.12	0.080	0.75	0.009	0.43
P (1–4)	Healthy	—	1.52	0.010	0.029	0.044	0.63	0.009	0.37
P (1–4)	Original	—	1.32	0.024	0.027	0.040	0.49	0.012	0.37
B (1, 3, 4)	Pitted	38	2.42	0.033	0.14	0.074	0.80	0.018	0.49
B (1, 3, 4)	Healthy	—	1.72	0.011	0.029	0.044	0.70	0.021	0.43
B (2)	Pitted	67	2.57	0.032	0.15	0.080	0.94	0.023	0.42
B (2)	Healthy	—	1.84	0.016	0.030	0.043	0.74	0.020	0.40
B	Original	—	1.68	0.028	0.030	0.043	0.58	0.014	0.39

movement of potassium, sodium, and nitrogen took place. There were only three exceptions where the amount of nutrient was less in the affected tissue than in neighbouring apparently healthy tissue: for potassium with sodium tree 3, for sodium with boron trees 1, 3, and 4, and for nitrogen with potassium treatment.

The largest movements, in absolute figures, have occurred with total ash, potassium, and nitrogen, but the largest relative movement, which is really outstanding, has been with magnesium. Here at both Annesbrook and the Moutere Hills, and also in the commercial orchards, there was nearly a fourfold concentration compared with the neighbouring tissue, and this was even greater when comparison is made

with the "original" figures. Among the other constituents, total ash has increased by about half, calcium has doubled or trebled, phosphorus has doubled, while potassium and nitrogen increased in general to a relatively small degree, and sodium increased by about a fifth. It is apparent therefore that there have been great differences in the movements of the several constituents from the bulk of the tissue into those areas which had become necrotic.

When the "original" data are compared with those of the corresponding apparently healthy tissue bordering the pitted areas it is found that this healthy tissue is higher in all constituents in the Annesbrook fruit, and always higher in total ash, usually in phosphorus, potassium, sodium, and nitrogen, about the same in magnesium, but markedly lower in calcium, in the Moutere Hills fruit.

In Table 2 there are four treatments, calcium, magnesium, sodium, and boron, which have two sets of data covering widely different degrees of incidence of pitting. When the data for pitted tissue for each degree of incidence are compared, the more severely affected fruit shows the higher total ash, magnesium, potassium, sodium, and phosphorus, contents; nitrogen does not show consistent variations. Calcium content of the more severely affected fruit is the lower in all four pairs of data. It is interesting that the calcium content of the nominally healthy tissue is so much lower than the "original" figures. This suggests that calcium is drained from the immediate surroundings of the pitted tissue and because it is not so mobile as other constituents it is not maintained to the same relative degree. Magnesium in the healthy tissue is usually the same as in the "original" sample. On the other hand total ash and potassium are higher in the nominally healthy tissue, while phosphorus and nitrogen are not consistent in their variations from the "original" figures. Phosphorus in the affected tissue is however consistently nearly double that of the neighbouring healthy tissue.

One further remarkable fact remains for comment: this is the constant level of magnesium (0.14%) and of phosphorus (about 0.09%) in the affected tissues of the Moutere Hills fruit; the same magnesium and (average) phosphorus figures apply to the Annesbrook fruit. Garman and Mathis (1956) found, on the average for five samples of Baldwin apples, that affected tissue contained 0.13% magnesium and 0.09% phosphorus in March, rising to 0.28% and 0.12% respectively in December. The Nelson fruit was not held long enough for similar comparisons to be made. These data suggest that continuing movement of nutrients into the necrotic tissue may take place even after necrosis has occurred.

Sugars and Starch

The data obtained for samples from commercial orchards suggested that the fructose content of pitted material was somewhat lower than that of healthy tissue. Compared with the "original" figures glucose had increased during storage. That there is a marked difference in fructose content between affected and healthy tissue has not been confirmed in the 1959 season at either Annesbrook or the Moutere Hills orchard as the data of Tables 3 and 4 show, but an increase in

glucose content during storage has been found again. In Tables 3 and 4 the glucose in the tissues after storage is three to four times that at harvest; moreover the glucose is higher in the pitted as against the neighbouring healthy tissue in two out of three examples at Annesbrook and in all eleven at the Moutere Hills. But when the figures are taken for the four within-treatment comparisons glucose is higher in the high-pitting fruit in only two of the four. Fructose is higher in the pitted tissue in two out of three at Annesbrook and in three out of eleven comparisons at the Moutere Hills, and is higher in pitted tissue from the more badly affected fruits in two out of four examples in the latter orchard. Total reducing sugars are higher in all three treatments at Annesbrook and in seven out of eleven comparisons in the other orchard. Sucrose is lower in the pitted than in the accompanying healthy material in all at Annesbrook and in all, except one set, of the Moutere Hills data; for the within-treatment comparisons sucrose is lower in three of the four samples from the more badly affected fruits.

TABLE 3. Percentages of Sugars and Starch on D.M. Basis of Affected and Healthy Tissue from Fruits from Annesbrook

Treatment	Status	Bitter Pit %	Glucose %	Fructose %	Total Reducing Sugars %	Sucrose %	Starch %
Na (1–3)	Pitted	40	13.1	35.9	49.0	5.2	0.70
Na (1–3)	Healthy	—	9.5	36.8	46.3	5.4	0.14
Na (1–3)	Original	—	4.6	24.8	29.4	17.1	—
K (3)	Pitted	92	7.2	33.0	40.2	2.6	0.82
K (3)	Healthy	—	10.3	28.9	39.2	3.7	0.24
K (3)	Original	—	4.6	20.9	25.5	12.3	—
P (1–3)	Pitted	42	13.3	34.4	47.7	2.2	0.91
P (1–3)	Healthy	—	8.1	34.3	42.4	3.0	0.19
P (1–3)	Original	—	5.0	19.2	24.2	13.5	—

As seen in Tables 3 and 4 the composition of the nominally healthy tissue is markedly different from the average tissue at harvest. The increase in glucose in the former has already been noted. "Original" sucrose at Annesbrook was three to four times as high as that at time of removal of the fruit from the cool store. In the Moutere Hills fruit the apparently healthy tissue approximates in sucrose content to the "original" fruit. Continued manufacture of sucrose after removal from the tree has been noted by others (Kidd *et al.* 1952) and has been related to the disappearance of starch from the tissues. But fructose and total reducing sugars contents in all cases at both orchards were much lower at harvest than at removal from storage. Hydrolysis of starch would not provide all the increased sugars contents, so that

TABLE 4. PERCENTAGE OF SUGARS AND STARCH ON D.M. BASIS OF AFFECTED AND HEALTHY TISSUE FROM FRUITS FROM MOUTERE

Treatment	Status	Bitter Pit %	Glucose %	Fructose %	Total Reducing Sugars %	Sucrose %	Starch %
Cont. (1–4)	Pitted	27	13.9	39.5	53.4	5.5	1.37
Cont. (1–4)	Healthy	—	10.9	39.7	50.6	13.7	0.05
Cont. (1–4)	Original	—	4.8	22.5	27.3	14.2	—
Ca (1–3)	Pitted	17	12.4	36.2	48.6	8.7	0.95
Ca (1–3)	Healthy	—	10.8	41.6	52.4	17.1	0.05
Ca (4)	Pitted	68	14.7	45.3	60.0	1.0	0.93
Ca (4)	Healthy	—	10.9	40.8	51.7	15.9	0.13
Ca	Original	—	3.9	27.4	30.3	14.5	—
Mg (1–3)	Pitted	35	14.2	41.8	56.0	7.3	1.33
Mg (1–3)	Healthy	—	10.2	39.2	49.4	16.3	0.27
Mg (4)	Pitted	82	13.8	38.5	52.3	6.0	1.08
Mg (4)	Healthy	—	10.6	40.8	51.4	12.9	0.12
Mg	Original	—	3.7	25.7	29.4	14.7	—
K (1–4)	Pitted	66	15.2	40.7	55.9	8.7	1.07
K (1–4)	Healthy	—	11.9	41.7	53.6	14.8	0.12
K (1–4)	Original	—	4.4	26.1	30.5	16.5	—
Na (1, 2, 4)	Pitted	64	13.8	34.4	48.2	10.8	1.73
Na (1, 2, 4)	Healthy	—	11.9	38.6	50.5	15.1	0.12
Na (3)	Pitted	26	12.1	32.6	44.7	15.7	N.D.
Na (3)	Healthy	—	10.9	38.7	49.6	11.2	0.13
Na	Original	—	4.3	26.4	30.7	14.9	—
P (1–4)	Pitted	28	14.6	36.5	51.1	7.2	1.05
P (1–4)	Healthy	—	11.9	38.2	50.1	14.7	0.20
P (1–4)	Original	—	4.7	27.3	32.0	14.8	—
B (1, 3, 4)	Pitted	38	15.1	37.3	52.4	6.4	1.04
B (1, 3, 4)	Healthy	—	11.5	35.5	47.0	11.6	0.06
B (2)	Pitted	67	12.0	29.8	41.8	14.4	1.11
B (2)	Healthy	—	10.8	33.9	44.7	23.6	0.15
B	Original	—	5.7	23.9	29.6	14.4	—

sugars must be metabolised from some other source within the apple during storage.

At Annesbrook increased incidence of pitting is associated with lowered fructose and total reducing sugars contents of the tissues, but no such relationships are in evidence for the Moutere Hills fruit. In the latter, neither glucose nor sucrose content bears any relationship to the incidence of bitter pit.

Starch content of the fruit at harvest has not been determined, so that in Tables 3 and 4 data for this constituent are given only for the affected and its accompanying healthy tissue. At Annesbrook the two

averages are 0.81% and 0.19% respectively. For the Moutere Hills samples they are 1.17% and 0.13%. There are thus very great differences in the starch contents of the two types of tissue. Affected tissue from a given treatment has about the same percentage of starch even though the incidence of pitting in the fruit from which the samples came might be much greater in one tree than in the others. The level of starch in the apparently healthy tissue does not seem to be consistently related to the degree of pitting of the fruit.

DISCUSSION

Because the data for sugars and starch contents do not suggest relationships between the levels of these constituents and incidence of bitter pit in the fruit, attention will be transferred to the minerals data of Tables 1 and 2.

At both Annesbrok and the Moutere Hills there are very distinct relationships between the degree of pitting of the fruits and the total ash contents of the affected and healthy tissues, the pitting being more severe with increasing ash content. The equations connecting ash content (y) with percentage of pitted fruits (x) in the Moutere Hills fruit are as follows:

$$y \text{ (healthy tissue)} = 0.0042\,x + 1.55$$

$$y \text{ (pitted tissue)} = 0.0064\,x + 2.29$$

Correlation coefficients have been calculated for these several quantities: for the healthy tissue $r = 0.77$ with $P = 1\%$, and for the affected tissue $r = 0.875$ with $P = 0.1\%$. There are thus quite definite connections between increased ash content and increased severity of pitting when discoloured tissue and its neighbouring apparently healthy tissue are analysed.

TABLE 5. AVERAGES AND RANGES OF VALUES OF SEVERAL RATIOS BETWEEN CONSTITUENTS OF PITTED, HEALTHY, AND "ORIGINAL" TISSUES OF FRUITS, 1959

Orchard	Tissue		Mg/Ca	Mg/P	K/Mg	K/Ca	K/N
Annesbrook	Pitted	Average	3.75	1.67	6.4	23.5	1.30
	Pitted	Range	3.50–4.24	1.27–2.15	4.6–9.1	19.4–31.7	0.97–1.81
	Healthy	Average	1.83	0.60	24.7	45.1	1.17
	Healthy	Range	1.25–1.95	0.52–0.65	22.0–30.0	38.5–53.7	1.10–1.20
	Original	Average	1.25	0.67	20.5	30.9	0.99
	Original	Range	1.44–1.81	0.64–0.69	17.7–24.8	25.5–38.8	0.88–1.13
Moutere Hills	Pitted	Average	4.13	1.61	6.3	26.1	1.68
	Pitted	Range	3.14–5.19	1.26–1.89	5.1–8.3	17.7–43.0	1.25–2.24
	Healthy	Average	2.14	0.83	23.8	50.2	1.55
	Healthy	Range	1.50–2.90	0.47–1.93	17.0–29.3	30.7–63.6	0.96–1.88
	Original	Average	1.07	0.64	19.9	23.1	1.30
	Original	Range	0.74–1.26	0.54–0.70	18.1–21.9	20.4–28.3	1.00–1.59

The data for the whole samples at picking also follow the rule of high ash content as an accompaniment of high incidence of bitter pit but the relationships are not as precise as with the isolated tissues.

Values of a number of ratios of the percentages of nutrients in the dry matter of the affected and accompanying healthy tissues, and for the average composition of the peeled fruits, are given in Table 5 for both Annesbrook and Moutere Hills orchards. A most striking fact is the wide differences in the ratios for the different classes of tissue. Thus in the pitted tissue the ratio Mg/Ca of about 4 is twice as high as in the nominally healthy tissue, and 3–4 times as high as the "original" tissue. Even more striking are the differences in the values of the K/Mg ratios; here the pitted tissue shows the relatively low values of a little over 6, while the other two tissues have values of 24 and 20 respectively. The ratio K/Ca also exhibits wide variations for the three classes of tissue. The table also shows that the individual ratios varied somewhat for a given tissue. This prompts the inquiry as to whether these variations are correlated with the incidence of bitter pit. A summary of the relationships is given in Table 6.

TABLE 6. CORRELATIONS BETWEEN INCIDENCE OF PITTING AND THE VALUES OF RATIOS BETWEEN CONSTITUENTS OF FRUITS, 1959

Orchard	Tissue	Mg/Ca	Mg/P	K/Mg	K/Ca	K/N
Annesbrook	Pitted	nil	negative	positive	positive	positive
	Healthy	nil	nil	positive	positive	nil
	Original	positive	nil	positive	positive	positive
Moutere Hills	Pitted	positive	nil	nil	positive	positive
	Healthy	nil	nil	nil	nil	nil
	Original	nil	nil	nil	positive?	nil

For the Annesbrook fruit, severity of pitting increased with increase in the values of the ratios K/Mg, K/Ca, and K/N with all three types of tissue. The values of the ratio Mg/P decreased with increase in pitting for the pitted tissue only; there were no relationships for the other two tissues. On the other hand only the "original" tissue showed a connection, a positive one, between value of the ratio Mg/Ca and increased pitting of the fruit on storage.

With the Moutere Hills fruit there were singularly few relationships between the values of the several ratios and incidence of bitter pit in the fruit. No definite relationships existed for the healthy and "original" tissues with any of the ratios. For the pitted tissues there were close connections, especially so for the first one, between increasing values of the ratios Mg/Ca, K/Ca, and K/N. It will be noted that the last two ratios were of particular interest with the Annesbrook fruit.

For diagnostic purposes it would have been particularly valuable had more connection been found between incidence of pitting and the

numerical size of the ratios. Although the Annesbrook fruit shows closer relationships than the Moutere Hills material, yet by reason of the larger selection of data from the latter it would seem unwise to attach too much importance to the Annesbrook figures.

Acknowledgment

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BLACK ROOT DISEASE OF RADISHES CAUSED BY *Aphanomyces raphani* Kendr.

By H. T. Wenham, Massey Agricultural College, Palmerston North

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Summary

Black root of radishes caused by *Aphanomyces raphani* Kendr. is widely distributed in the Manawatu. The disease has not previously been reported in New Zealand.

Symptoms are severe stunting, with blackening and deformity of roots.

The morphology, colony characteristics, and reproduction of the causal fungus are described.

Sexual reproduction has been observed on PDA, cornmeal agar, and oatmeal agar.

The pathogen remains viable for only a few weeks on PDA, but survives for several months on cornmeal agar.

In field tests the variety French Breakfast proved highly resistant

Introduction

A disease of radishes (*Raphanus sativus* L.) characterised by severe blackening and deformity of roots has been known in the Manawatu for several years and commonly regarded by commercial growers as indicative of a soil deficiency of boron. Studies have shown this condition to be in fact black root, a soil-borne disease caused by the fungus *Aphanomyces raphani* Kendr. and not previously reported in New Zealand. A survey of home-garden and commercial radish crops conducted over three years has revealed the disease to be widespread in the Manawatu, in several instances causing severe losses.

Radish black root was first recorded in Illinois by Barrett (1912) who identified the casual fungus as *Aphanomyces laevis* de Bary. Edson (1915) reported a disease of radishes with similar symptoms caused by an apparently undescribed fungus which he named *Rheosporangium aphanidermatus*. This organism was later placed in the genus *Pythium* (Fitzpatrick 1923) and thereafter black root of radishes was generally attributed to *Pythium aphanidermatum* (Edson) Fitz. Kendrick (1927a) studied the disease in Iowa and Indiana and determined the true cause to be a species of *Aphanomyces* quite distinct from *A. laevis* de Bary, which he named *Aphanomyces raphani* Kendr. In no instance did Kendrick find *Pythium aphanidermatum* associated with diseased roots and all inoculation attempts using pure cultures of this latter fungus were unsuccessful.

The disease has been studied in Germany by Herold (1952) who was unable to demonstrate a correlation between black root and boron deficiency.

A. raphani has been reported as causing serious losses in radish crops in Germany (Boning 1932), Canada (Conners *et al.* 1957), and

Australia (Mr R. Pengelly pers. comm.). The disease has not been recorded in the United Kingdom (Dr F. Joan Moore pers. comm.).

Symptoms

Black root is characterised by severe stunting and blackening of the fleshy root tissues. Lesions on younger roots may restrict growth in that area causing pronounced girdling and deformity as diseased tissues fail to keep pace with root growth (Fig. 1). In the early stages

Fig. 1.—Radish roots showing symptoms of black-root disease caused by *Aphanomyces raphani* Kendr.

the darkened areas are small, superficial, and located in the immediate vicinity of the point of emergence of secondary roots. Such areas frequently enlarge and coalesce as the root develops so that areas two inches in diameter may be involved. By this time the pathogen has usually penetrated deeply causing the tissues to be partially blackened. Dark, lead-coloured strands of infected tissue frequently extend irregularly through neighbouring healthy white tissues. In the advanced stages the internal root tissues are uniformly blackened and collapsed (Fig. 2).

Isolation and Pathogenicity

A. raphani is difficult to isolate in pure culture on account of saprophytic bacteria which are almost invariably associated with diseased host tissues. Infected pieces of root plated to prune agar, malt agar, and acidified PDA, failed to yield the fungus although the relatively low pH of the media largely suppressed bacterial growth. Best results were obtained by removing small pieces of diseased tissue from cut infected roots, washing for 6 hours in running tap water and plating on water agar. Sparse phycomycetous hyphae usually developed from up to 15% of such plated tissue pieces. Early experience showed

Fig. 2.—Longitudinally cut root in the advanced stage of collapse.

that bacteria were still frequently associated with this mycelium. Thereafter pure cultures were obtained using the Van Tieghem cell method for separating fungi from bacteria as described by McKeen (1949).

Several methods were used successfully to prove pathogenicity of the isolated fungus. However the Hickman zoospore method (Hickman 1953) was found the most convenient and was used in the majority of inoculation experiments. This method involved immersing roots of 15-day-old disease-free seedlings in a zoospore suspension of the fungus for 24 hours, and then pricking out in boxes of sterilised soil. The inoculum was prepared by flooding 6-day-old PDA cultures with sterile tap water and holding at room temperature for two days. Plants were pulled and washed 37 days after pricking out and found to exhibit symptoms typical of field-diseased plants. Plants of the control series, which had been pricked out after immersing the roots in sterile tap water, were invariably disease free.

The fungus was re-isolated to water agar from roots of the inoculated series following the method earlier described.

THE PATHOGEN IN PURE CULTURE

A. raphani grew well on PDA, cornmeal agar, oatmeal agar, and nutrient agar, but did not grow on prune agar, lima-bean agar, or malt agar. Transfers to water agar from young actively growing PDA cultures were rarely successful. On PDA, maximum growth was at 23°C with only slight growth at 8° and 32°C. The surface of inoculated plates 90 mm in diameter held at 23°C was usually covered within 9 days. Such colonies consisted of a cream-coloured, moist, dense, very tough mycelial thatch free from aerial hyphae.

Considerable difficulty was at first experienced in maintaining stock cultures. Cornmeal agar was found to be the most satisfactory

medium, subcultures to PDA being readily prepared from stock slopes held for 100 days at 23°c. On PDA the fungus remained viable for much shorter periods, in no instance for longer than 29 days. McKeen (1952) records *Aphanomyces cladogamus* Drech. as remaining viable on PDA for only a few weeks whereas cornmeal agar cultures were kept alive for six or seven months between transfers.

Morphology of the Pathogen

The vegetative hyphae are hyaline, non-septate, and profusely branched, and ranged from 5.5 to 12 μ in diameter. The details of asexual reproduction were studied by transferring agar squares from the edge of actively growing colonies to petri dishes of sterile water. Within 6 hours hyphae destined to function as zoosporangia had grown out into the water. They were long and sparingly branched, and differed from vegetative hyphae in being occasionally spirally branched and tapering slightly towards the tip. Zoospores were produced in a single row in the zoosporangium by segmentation of the contents. They were cylindrical and 5 to 25 μ in length. The tips of the hyphae dissolved and each then functioned as an evacuation tube, the protoplasmic units flowing rapidly out into the water where they instantly assumed a spherical shape to become non-motile zoospores. At this stage the appearance was that of clumps of encysted zoospores grouped at the orifice of the evacuation tubes (Fig. 3). The zoospores soon became free, drifted in the water for a while and then settled on the bottom of the petri dish in such large numbers as to give a cloudy, scum-like appearance. Most zoospores passed into a motile spore stage within 6 hours of encystment. They swam actively for approximately 3 hours, came to rest, and almost immediately germinated by a germ tube (Fig. 4).

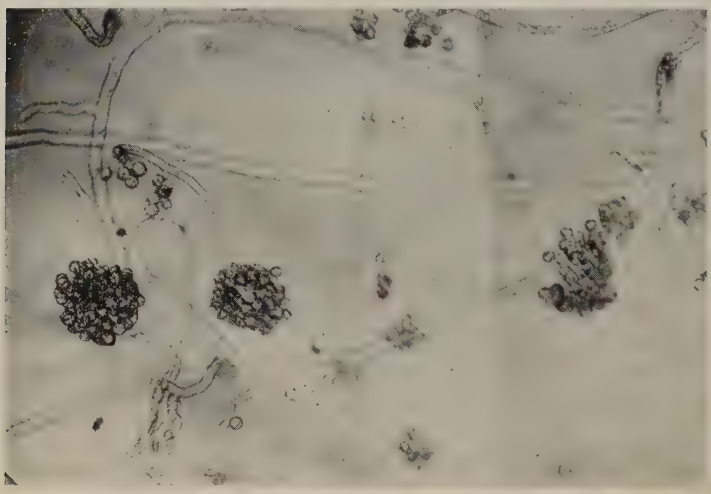

Fig. 3.—Groups of encysted zoospores at tips of zoosporangia.

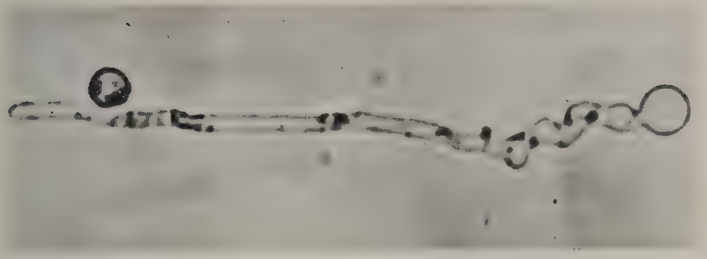

Fig. 4.—A zoospore germinating after encystment.

The sexual apparatus of the causal fungus was located in severely diseased roots, but morphological details were more readily observed on cornmeal and oatmeal agar where oogonia were produced in abundance within 10 days of inoculation. Sexual reproduction did not occur on either peameal agar or nutrient agar and only an occasional oogonium was observed on PDA cultures.

Oogonia were terminal on short branches, spherical, smooth, 23 to 37 μ (av. 33.4 μ) in diameter, and when mature possessed a thick wall, the inner surface of which was wrinkled. Oospores were formed singly in oogonia, were smooth, 15 to 32 μ (av. 23.6 μ) in diameter, and usually contained a single prominent oil globule. Antheridia were mostly of diclinous origin but a few were observed arising from the same hypha bearing the oogonial stalk. Usually two clavate antheridia became attached to each oogonium. At the distal inflated part they averaged 10.5 μ in diameter.

CONTROL

Kendrick (1927b) has shown radish varieties to vary considerably in their susceptibility to black-root disease. Field observations in the Manawatu suggested variation in the relative susceptibility of the four varieties commonly grown in this area, namely White Icicle, Long Scarlet, French Breakfast, and Mixed Turnip. During September 1958 and January 1959 seed of the above varieties was sown on five properties that had previously produced diseased crops. Forty days later plants were pulled, washed, and assessed for infection.

In each planting the variety French Breakfast proved highly resistant, roots being well developed and practically free from lesions. The other three varieties in all plantings were severely stunted, blackened, and deformed. The extent to which the disease induces stunting was indicated by the occasional plant that had escaped infection. In one plot the average weight of disease-free roots of the variety White Icicle (the most susceptible variety) was 3.8 times greater than diseased roots.

Where it is desired to sow radishes in soil that has previously yielded diseased plants only the variety French Breakfast should be considered. Alternatively, soils with a long history of black-root disease should be spelled from radishes for several seasons. The pathogen is

perpetuated in infected roots by oospores which on disintegration of the roots are released to the soil and there serve as primary inoculum (Boning 1932). Since radish is the only known host for *A. raphani*, a gradual reduction in the inoculum potential will result if this crop is not grown for several years.

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FACTORS INFLUENCING SURVIVAL OF STRAINS OF RYEGRASS DURING THE SUMMER

By Renate Lucanus, K. J. Mitchell, G. G. Pritchard,* and D. M. Calder,† Department of Scientific and Industrial Research, Palmerston North

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Summary

The influence of levels of temperature, soil moisture, soil nitrogen status, and of light intensity on the survival of New Zealand strains of ryegrass (*Lolium* spp.) was examined.

It was found that not only was the survival of plants reduced by soil moisture stress (pF 3.4–4.2 compared with pF 2.6–3.0) and high temperature (80°F compared with 65°F), but also that survival under the low moisture or high temperature condition was much less when there was a high level of nitrogen in the soil. The various effects were additive. In general the survival of plants of perennial ryegrass was significantly better than that of plants of short-rotation and Italian ryegrass.

A reduction of light intensity to 20–25% of full daylight also adversely affected survival, particularly when it occurred in conjunction with high temperatures.

Introduction

During the summer, plants in a pasture frequently experience both a shortage of soil moisture and high temperatures. As a result there may be a considerable reduction in the density of a sward, and in some cases a species or strain may be eliminated almost completely during the course of a summer.

Problems of drought resistance in plants and the manner in which it is influenced by other environmental conditions have been considered on many occasions (e.g. Heyne and Brunson 1940; Carroll 1942; Julander 1945; Iljin 1957). Although Levitt (1951) concluded that frost, drought (i.e. desiccation), and heat resistance, are basically similar, the relative importance of the various physiological mechanisms, and of associated environmental factors such as light intensity which condition resistance, probably differ in various species and strains. Recently significant advances in the understanding of biological mechanisms for both heat and drought resistance have been made (Bonner 1957; Langridge and Griffing 1959).

Accordingly, before commencing studies of the physiological and biochemical mechanisms which govern the survival of pasture species during summer, it was desired to obtain information on the influence of factors believed to be of major importance. The work reported here examined the influence not only of soil moisture and of temperature but also of nitrogen status and of light intensity on the survival of plants of various strains of ryegrass (*Lolium* spp).

* Present address: Queen Mary College, University of London, England.
† Present address: Massey Agricultural College, Palmerston North.

Experimental Methods

Influence of Genotype, Temperature, Soil Moisture, and Soil Nitrogen Status

There were two trials: a preliminary trial carried out during the summer and early autumn months of 1956 and a main experiment during the same period in 1957.

Genotype. Perennial ryegrass, short-rotation ryegrass and Italian ryegrass were used in both the preliminary and the main trial and, in addition, false perennial ryegrass (Levy and Davies 1930a, b) was included in the preliminary trial. The strains of ryegrass used in both the preliminary and the main trial were:

(a) N.Z. perennial ryegrass (*Lolium perenne* L.);

(b) N.Z. short-rotation ryegrass (*L. perenne* L. × L. *multiflorum* Lam.);

(c) N.Z. Italian ryegrass (*L. multiflorum* Lam.).

All plants were supplied by the plant-breeding section of Grasslands Division, Department of Scientific and Industrial Research, from increases of the latest nucleus isolation of each strain as available at the time of the experiment.

In mid December of each year substantial portions were removed from single plants growing in the field. These portions were carefully broken up and uniform three-tiller cuttings selected for planting into containers. Twelve cuttings were established in each container.

At the time of selection in the field the plants were nearing the end of their flowering period. Although a few flower heads appeared in the first weeks after transplanting, growth while the various treatments were under way was predominantly vegetative.

Eight treatment combinations were applied to each genotype. These were: high and low temperature, high and low soil moisture and high and low soil nitrogen status.

Temperature. For the high-temperature treatment in each trial half the plants were placed in a warm glasshouse where the temperatures averaged about 80°F and occasionally rose to well over 100°F: for the low-temperature treatment the other half of the plants were placed outside at a mean temperature of approximately 65°F. The plants outside were protected from natural rainfall by glass covers placed several feet above them so that there was free circulation of air around the plants and the temperatures of the plants and soil were not raised by the presence of the glass. The glass also served to reduce the light received by the plants outside to approximately the same level as that received by the plants in the glasshouse.

Moisture. Within each genotype and temperature group the plants in half of the containers were given high-moisture conditions and the other half low moisture. In the high-moisture treatment, where the aim was to maintain adequate but not excessive moisture in the soil, pF was held between 2.6 and 3.0. In the low moisture series the

soil was allowed to dry out gradually until the moisture level was close to or at wilting point. The containers were then lightly watered from below to bring the pF back to 3.2–3.5 before allowing the soil to commence drying out again. In the main trial (1957) a gypsum block was placed in each container to give direct measurement of pF. This gave better regulation of moisture levels than occurred in the preliminary trial, in which eye judgment was used and some excessive drying-out occurred.

Nitrogen. For all plants 5.2 g of blood and bone fertiliser containing 0.4 g of nitrogen (equivalent to approximately 2.5 cwt per acre of sulphate of ammonia) was mixed into the soil (approximately 25 lb of silt loam soil) of each container, at the time of planting. This was intended only to provide a supply of nitrogen to assist establishment. For the low-nitrogen treatment it was the only nitrogen fertiliser that the plants received during the trial.

The plants in the high-nitrogen treatments received, eight days before commencement of temperature and moisture treatments described, nitrogen fertiliser sufficient to provide 1.6 g of nitrogen per container (equivalent to approximately 10 cwt per acre of sulphate of ammonia), and a further application of 0.8 g of nitrogen per container (equivalent to approximately 5 cwt per acre of sulphate of ammonia) four weeks after commencement of the temperature and moisture treatments. Two-thirds of this nitrogen came from urea and one-third from blood and bone fertiliser.

In the preliminary trial there were two containers for each of the genotype treatment combinations described above. In the main trial there were four containers for each of these genotype treatment combinations, but two out of each group of four containers received 3.5 g of muriate of potash at the time of planting (equivalent to approximately 4 cwt per acre of muriate of potash). Reports in the literature indicated that with some species potash level apparently influenced the drought tolerance of plants (Collings 1948; Scharrer 1953). Hence the effect of supplementary potash was checked even although the soil used in the experiments was known to be high in potash. As there was generally little effect of potash, this treatment is disregarded in considering the main body of results.

Plants were cut periodically at a height of 1 in. above ground level. At intervals, surviving plants were counted, and at the end of the experiment all containers were placed outside, given adequate moisture, and left for two months to determine whether plants that were apparently dead would show regrowth. The final count of surviving plants was then taken.

Influence of Light Intensity

The effect of a substantial lowering of light intensity was examined in a trial carried out alongside the 1956 nitrogen/soil-moisture trial. It used the same sets of temperature conditions. At both levels of temperature the plants received either 60–70% of the summer daylight (termed "full light") or 20–25% of summer daylight (termed "shaded").

A cover of a single layer of muslin was used with both low-temperature treatments to compensate for the reduction in light intensity inside the glasshouse used for the high-temperature treatment. At both temperature levels a double layer of scrim was used to give the reduction of light intensity to 20–25% of daylight.

Except for the omission of false perennial ryegrass, the ryegrass strains used were the same as those in the 1956 nitrogen/soil-moisture trial. Plants were established in glazed pots and grown with an adequate but not extremely high level of soil moisture and nitrogen. All plants received the same defoliation treatment. Percentages of surviving plants were recorded.

RESULTS

Influence of Genotype, Temperature, Soil Moisture, and Nitrogen

Results from the preliminary experiment are presented in Table 1 and those of the main experiment in Tables 2 and 3 and in Fig. 1.

Although the low-moisture treatments were rather more drastic in the preliminary experiment, with the result that at high temperature and low moisture no plants survived, there was close agreement between the results of the two experiments. Not only was the survival of the plants reduced by low moisture and by high temperature but

TABLE 1. PERCENTAGES OF SURVIVING PLANTS AT THE END OF THE PRELIMINARY TRIAL (Feb. 1956)

Strain	Nitrogen Level	Low Temperature		High Temperature	
		High Moisture	Low Moisture	High Moisture	Low Moisture
Perennial ryegrass	High	100	64 ± 5	100	—
	Low	100	98 ± 1	100	—
Short-rotation ryegrass	High	98 ± 1	44 ± 5	4 ± 2	—
	Low	100	98 ± 1	94 ± 2	—
Italian ryegrass	High	100	10 ± 3	16 ± 4	—
	Low	94 ± 2	72 ± 5	94 ± 2	—
False perennial ryegrass	High	98 ± 2	16 ± 5	56 ± 7	—
	Low	98 ± 2	70 ± 1	60 ± 7	—

TABLE 2. PERCENTAGES OF SURVIVING PLANTS AT THE END OF THE MAIN TRIAL (March 1957)

Strain	Nitrogen Level	Low Temperature (65°F)		High Temperature (80°F)	
		High Moisture (pF 2.6–3.0)	Low Moisture (pF 3.5–4.3)	High Moisture (pF 2.6–3.0)	Low Moisture (pF 3.5–4.3)
Perennial ryegrass	High	100	65 ± 11	67 ± 10	19 ± 8
	Low	98 ± 1	94 ± 4	100	67 ± 9
Short-rotation ryegrass	High	100	31 ± 10	73 ± 9	15 ± 6
	Low	100	96 ± 2	96 ± 3	48 ± 10
Italian ryegrass	High	96 ± 2	46 ± 11	56 ± 11	—
	Low	90 ± 4	79 ± 9	56 ± 10	25 ± 8

also ability to survive at either low moisture or high temperature was lower if the nitrogen status of the soil was high (Tables 1 and 2)

In general, survival was higher with perennial ryegrass than with short-rotation ryegrass and Italian ryegrass.

Fig. 1 shows the percentage survival of the 48 plants of short-rotation ryegrass in each treatment during the 1957 experiment. Data are presented only for short-rotation ryegrass as representative of the main trends which occurred. Under high temperature and low moisture, death of plants commenced early in the experiment. It was also quickly evident that the survival of the plants at the high nitrogen level was much poorer than at the low nitrogen level, except where they were growing under low temperature and high moisture.

Production of herbage per plant was initially higher in all moisture and temperature treatments from plants with high nitrogen status as compared with those with low nitrogen status. However, under low moisture or high temperature these differences soon disappeared, and under these conditions production per surviving plant became similar irrespective of nitrogen status in the soil.

In general, the addition of potash fertiliser did not improve the ability of plants to survive. However, there was one instance where positive results were possibly present (Table 3). Under low moisture and low temperature the survival of perennial ryegrass was significantly better with the addition of potash fertiliser and that of short-rotation ryegrass possibly so (at 10% level). It is under similar temperature conditions that high potassium status is reported to enhance drought survival (Collings 1948; Scharrer 1953).

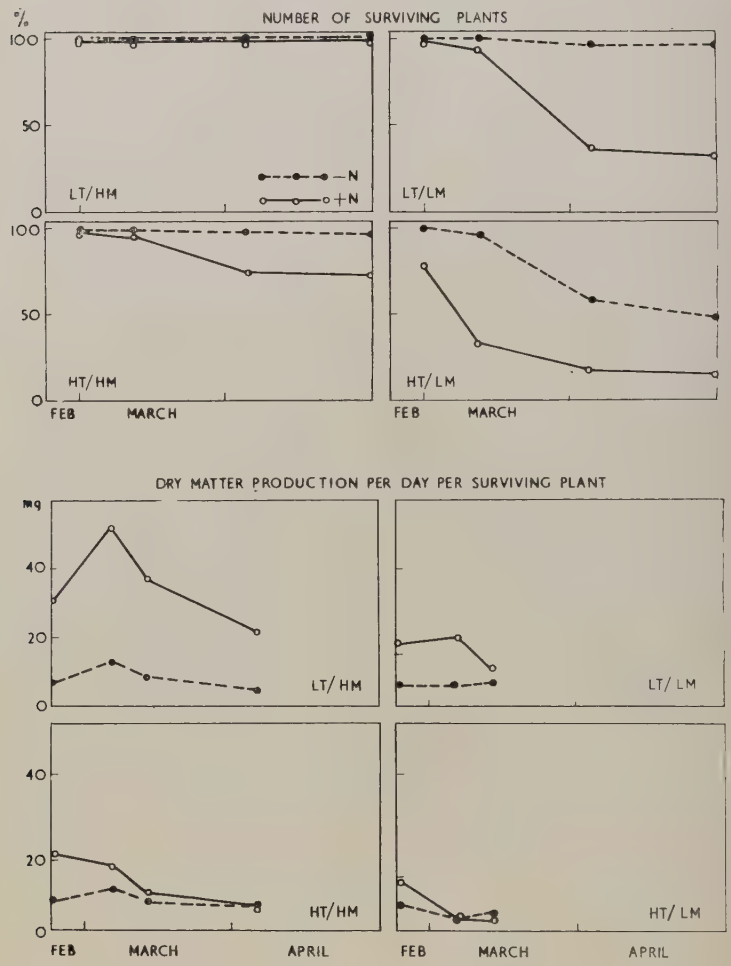

Fig. 1.—Survival and dry-matter production per day of short-rotation ryegrass during the course of the treatments (1957).

Influence of Shading

Even where plants were grown under favourable moisture and nutrient conditions, their ability to survive was reduced by shading to 20–25% of daylight (Table 4). This effect was more marked at the higher temperatures. Shade under these particular conditions had a significantly stronger injurious effect than heat alone.

TABLE 3. POTASH RESPONSE. PERCENTAGE OF SURVIVING PLANTS OF PERENNIAL RYEGRASS AND SHORT-ROTATION RYEGRASS AT LOW TEMPERATURE/LOW MOISTURE

Potassium Level	Perennial Ryegrass	Short-rotation Ryegrass
With potash	92 ± 5	71 ± 8
Without potash	67 ± 9	56 ± 10

TABLE 4. SHADING EFFECT. PERCENTAGES OF PLANTS SURVIVING AT DIFFERENT TEMPERATURE AND LIGHT LEVELS (FEB. 1956)

Strain	Low Temperature		High Temperature	
	Full Light	Shaded	Full Light	Shaded
Perennial ryegrass	100	54 ± 10	100	13 ± 7
Short-rotation ryegrass	100	46 ± 10	88 ± 7	4 ± 4
Italian ryegrass	100	8 ± 6	92 ± 6	17 ± 8
Average of 3 strains	100	36 ± 5	93 ± 4	11 ± 6

DISCUSSION

In the field it has been frequently observed that the survival of ryegrass during the summer is adversely affected by drought, or high temperature, or by shading such as occurs when clover is allowed to become dominant in a pasture. The finding that high nitrogen status in the soil also reduces survival under conditions of moisture and temperature stress is in some ways unexpected, for in many districts it has been found that raising soil fertility and hence nitrogen status gives a better growth of ryegrass at all seasons of the year. However, if regard is given to the comparative levels of nitrogen in the soil under the various sets of conditions, the two groups of observations are seen to be complementary. In past years the problem in the field has generally been to raise soil nitrogen status from a low to a moderate level. In the experiments reported here the high-nitrogen treatments would have produced a soil nitrogen status well above that generally met in the field and more comparable with that under a urine patch (Doak 1952) or in a stock camp.

The experimental results suggest that once an optimum of soil fertility and hence soil nitrogen status has been reached, attempts to

achieve even higher production of herbage from a pasture by continuing to build up soil nitrogen status may become self-defeating in districts which are subject to considerable temperature and drought stress and where pastures contain a high proportion of the strains of grasses used in these experiments. Excessive death of tillers during a hot or dry spell could occur, leading to a substantial opening of the pasture and to a reduction of its productivity. The results presented support the earlier findings by Dexter (1937) and Carroll (1942) that nitrogen fertilisation during summer reduced survival of plants of various graminaceous species in U.S.

The relatively good survival of plants of false perennial ryegrass which occurred in the preliminary trial is probably an indication of a physiological ability of the plants to withstand heat and moisture stress comparatively well once they have resumed vegetative growth after their period of flowering. The poor survival of plants of false perennial ryegrass in the field during summer is considered to be associated with their very strong change-over to the reproductive phase of development early in the summer (Cooper and Saeed 1949). The stronger that change-over, the more drastic are the reductions in the activity of roots and the formation of new tillers, both of which make it more difficult for plants to resume vegetative development, particularly under environmental stress.

The genotypes and treatments which gave poorer survival are associated with one or more of the following biochemical and morphological changes in the plant –namely, a higher percentage of total nitrogen and particularly of soluble nitrogen compounds (Bathurst and Mitchell 1958), a lower dry weight per unit area of leaf blade, a lower dry-matter percentage in the shoot (Mitchell 1956), and a decrease in quantity of root growth relative to shoot growth (Troughton 1957).

These gross biochemical and morphological changes induced by such treatments are more probably complementary to, or an indication of, changes in rates of synthesis or destruction of growth factors such as those that have been shown to regulate tolerance to high temperatures in various species and strains (Galston and Hand 1949; Bonner 1957; Langridge and Griffing 1959). Overall it would appear that in ryegrasses substances favouring tolerance to moisture stress and high temperatures are being synthesised most rapidly, or alternatively levels in the plant remain higher when the plants have a high "carbohydrate" status, as can come from either high light intensity or low nitrogen status.

Acknowledgments

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SEED DISINFECTION

XVI. A NOTE ON THE EFFECTS OF FUNGICIDES ON VIABILITY OF ONION SEED

By H. Jacks, Massey Agricultural College, Palmerston North

(*Received for publication, 19 November 1959*)

Tests were carried out to investigate the effect of various fungicides on viability of onion seed in storage, and on emergence of onion seedlings in glasshouse and outdoor soils.

Seed of the variety Pukekohe Longkeeper was used throughout the test. Fungicides were applied at 25% of seed weight, after seed was treated with a sticker (Jacks 1952). The following materials were used:

1. (50% thiram as Premasan)
2. (2.2% methyl mercuric dicyandiamide as Panogen)
3. (2.7% methylmercury oxinate as Metasol).
4. (1.5% methoxyethyl mercuric chloride as Ceresan)
5. (4% mercurous chloride as Calomel).
6. (98% chloranil as Spergon)
7. (10% quinone oxime benzoylhydrazone as Cerenox)
8. (50% dichlone as Phygon XL)
9. (65% zineb as Dithane Z.78).
10. (zinc mercury chromate).
11. (75% captan as Orthocide).
12. (50% copper oxychloride as Cuprox).
13. (90% cuprous oxide as Yellow Cuprocide).
14. (40% hexachlorobenzene as Anticarie).
15. (20% pentachloronitrobenzene as Tritisan).
16. (10% dinitrophenylthiocyanate as Nirit).
17. (2% ethyl mercuric chloride).

Seed was stored, in jars with their openings covered by muslin, at room temperature viz: 58 ± 3°F. Emergence tests were carried out in perlite moistened weight for weight with distilled water and placed in shallow dishes. Ten replications, each of 50 seeds, were used for each test, carried out immediately after treatment and after 2, 4, 8, 16 months, and two years of storage. Temperatures during test were the same as for storage. A general reduction in viability was recorded after two months and seeds failed to germinate after two years of storage. Treatments, 2, 4, 9, 10, and 17 gave significant (at 1% level) reduction of emergence as compared with checks, whilst treatments, 1, 3, 5, 7, 11, 13, 14, and 15 brought about a significant increase in seedling emergence. Calculations of the velocity coefficient (Jacks 1958) failed to show significant differences in the speed of emergence of various treatments.

Soon after treatment, seeds were sown in soil inoculated with *Pythium ultimum* Trow, and moisture was maintained at 50% water-holding capacity. There were ten replications each of 100 seeds per treatment. Glasshouse temperature during the test was 74 ± 6°F. Emergence was recorded 25 days after sowing. Emergence percentages were calculated on the basis of figures obtained from laboratory tests

carried out soon after treatment. Differences between treatments were not significant at the 1% level, but treatments 1 and 11 increased emergence at the 5% level of significance. The speed of emergence was increased by treatments 1, 5, 9, and 11 and reduced by treatments 6, 10, 12, 14, and 15.

In the field, five replications of 1000 seeds each were used per treatment. Records were taken one month after sowing. Soil temperature during the test was 59 ± 6°F and soil moisture 53 ± 8% water-holding capacity. Treatments 2 and 10 gave significant depression of emergence at the 1% level. Increase in emergence at the 5% level was brought about only by treatment 11, with treatment 1 just failing to reach significance.

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